THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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Science Library

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SERIAL LIST

A SERIAL list of the holdings of The Marine Biological Laboratory has been published as a separately bound supplement to The Biological Bulletin. This supplement lists with cross references the titles of journals in the Library; additional titles and changes are published annually. A few extra copies of the original list are still available. Orders may be directed to The Marine Biological Laboratory.

BOOKS AND WORLD RECOVERY

THE desperate and continued need for American publications to serve as tools of physical and intellectual reconstruction abroad has been made vividly apparent by appeals from scholars in many lands. The American Book Center for War Devastated Libraries has been urged to continue meeting this need at least through 1947. The Book Center is therefore making a renewed appeal for American books and periodicals—for *technical and scholarly books and periodicals in all fields* and particularly for *publications of the past ten years.* We shall especially welcome complete or incomplete files of the *Biological Bulletin.*

The generous support which has been given to the Book Center has made it possible to ship more than 700,000 volumes abroad in the past year. It is hoped to double this amount before the Book Center closes. The books and periodicals which your personal or institutional library can spare are urgently needed and will help in the reconstruction which must preface world understanding and peace.

Ship your contributions to the American Book Center, c/o The Library of Congress, Washington 25, D. C. freight prepaid, or write to the Center for further information.

Vol. 93, No. 1 August, 1947

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be

held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1946.

The accounts have been audited as in recent years by Messrs. Seamans, Stetson, and Tuttle, certified public accountants of Boston. A copy of their report is on file at the Laboratory office and inspection of it by Trustees or other Members of the Corporation will be welcomed.

The principal summaries of their report—The Balance Sheet, Statement of Income and Expense, and Current Surplus Account—are appended hereto as Exhibits A, B, and C.

The financial operations of 1946 may be summarized as follows:

I. Assets

1. Endowment Assets

As of December 31, 1946, the total book value of all the Endowment Assets, including the Scholarship Funds, was $977,199.30, an increase for the year of $10,427.14 due to profits on securities sold.

These Assets consisted of: marketable securities (bonds, preferred stocks, and common stocks) with a book or cost value of $889,416.18 and a market value of $934,042.19; six participations in mortgages on New York City realty costing $82,491.14, estimated by the Treasurer to have an actual value of $52,000,000; and $5,291.98 in uninvested principal cash.

The total current valuation of Endowment Assets was $991,334.17, $14,134.87 more than current book value, but $13,603.44 less than the actual value at the end of the previous year. This slight decline in market values during the year was due chiefly to lower prices for some of the common stocks.

2. Plant Assets

The total of Plant Assets at the end of the year was $1,312,963.70, or $17,884.05 less than at the end of 1945. New expenditures were more than offset by the depreciation charge of $25,314.41. In addition to heavy expenditures for repairs that were regarded as current expenditures, a total of $21,425.02 was paid out for improvements to buildings and new equipment that were regarded as capital

items. To take care of some of these payments $15,000 of the Reserve Fund was used, leaving a balance in the Reserve Fund of $15,859.89, a substantial portion of which has already been appropriated for repairs and equipment.

3. Current Assets

At the end of the year Current Assets had increased by $7,881.53 to a total of $220,851.88. Accounts Payable were $8,411.64. Current Surplus had increased $6,022.47 to $202,360.31.

II. Income and Expenditures

Both income and expense totals were much larger than in recent years. Total Income was $227,104.26. Total Expense, including $25,314.41 Reserve for Depreciation, was $234,537.13, leaving an accounting deficit of $7,432.87 compared with a surplus of $9,773.28 for 1945. The 1946 expenditures contained a number of unusual items such as $11,510.00 for "Special Repairs to the Brick Laboratory" and further "1944 Hurricane Damage Repairs" of $2,012.50.

On December 31, 1946, current cash on hand amounted to $28,135.30, but as in the case of the Reserve Fund, a portion of this was designated for further improvements and purchases already authorized.

Noteworthy in the income account are the contributions totalling $1,070.00 from the Marine Biological Laboratory Associates that were used for purchases of especially needed apparatus and a gift of $700 from Mrs. W. Murray Crane.

The total receipts of the Supply Department also attained what is believed to be a new high, $73,394.26. The expenses of the Supply Department, particularly the purchases of specimens from outside sources, also increased however to $62,527.53, so that the net gain from operations rose only moderately to $10,866.73.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1946

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 960,062.58	
Securities and Cash in Minor Funds		17,136.72	
			$ 977,199.30
Plant Assets:			
Land	$ 110,425.38		
Buildings	1,324,036.67		
Equipment	193,539.92		
Library	349,234.22		
	$1,977,236.19		
Less Reserve for Depreciation	696,981.38		
		$1,280,254.81	
Reserve Fund, Securities and Cash		15,859.89	
Book Fund, Securities and Cash		16,849.00	
			$1,312,963.70

Current Assets:			
Cash		$ 28,135.30	
Mortgage Note Receivable		2,500.00	
Accounts Receivable		30,114.98	
Inventories:			
Supply Department	$ 39,083.91		
Biological Bulletin	18,557.44	57,641.35	
Investments:			
Devil's Lane Property and Accounts Receivable	$ 48,055.31		
Gansett Property and Accounts Receivable	1,074.67		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Securities	20,795.00		
Retirement Fund Securities and Cash	12,757.24	95,382.22	
Prepaid Insurance		4,556.59	
Items in Suspense (Debits)		2,521.44	
			$ 220,851.88
Total Assets			$2,511,014.88

Liabilities

Endowment Funds:			
Endowment Funds	$ 958.871.15		
Reserve for Amortization of Bond Premiums	1,191.43		
		$ 960,062.58	
Minor Funds		17,136.72	
			$ 977,199.30
Plant Funds:			
Mortgage Note Payable		$ 5,000.00	
Donations and Gifts	$1,172,564.04		
Other Investments in Plant from Gifts and Current Funds	135,399.66	1,307,963.70	
			$1,312,963.70
Current Liabilities and Surplus:			
Accounts Payable		$ 8,411.64	
Items in Suspense (Credits)		$ 1,751.24	
Reserve for Repairs and Replacements		8,328.63	
Current Surplus		202,360.37	
			$ 220,851.88
Total Liabilities			$2,511,014.88

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1946

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 29,966.14		$ 29,966.14
Library Fund		6,000.47		6,000.47
Donations		2,170.00		2,170.00
Instruction	$ 11,788.90	9,030.00	$ 2,758.90	
Research	6,329.17	20,645.14		14,315.97
Evening Lectures	75.00		75.00	
Biological Bulletin and Membership Dues	10,500.17	12,863.15		2,362.98
Supply Department	62,527.53	73,394.26		10,866.73
Mess	32,567.63	30,069.72	2,497.91	
Dormitories	30,640.35	16,880.30	13,760.05	
(Interest and Depreciation charged to above 3 Departments)	(26,008.91)			26,008.91
Dividends, General Biological Supply House		17,272.00		17,272.00
Dividends, Other Investment Stocks		1,000.00		1,000.00
Rents:				
Bar Neck Property	873.71	6,000.00		5,126.29
Janitor House	21.45	360.00		338.55
Sale of Library Duplicates, Micro Film, etc.		331.61		331.61
Microscope and Apparatus Rental		1,011.47		1,011.47
Sundry Income		10.00		10.00
Interest on Mortgage Receivable		100.00		100.00
Maintenance of Plant:				
Buildings and Grounds	28,337.37		28,337.37	
Apparatus Department	8,267.02		8,267.02	
Chemical Department	3,859.74		3,859.74	
Library Expense	6,664.76		6,664.76	
Workmen's Compensation Insurance	435.15		435.15	
Truck Expense	157.11		157.11	
Bay Shore Property	172.32		172.32	
Great Cedar Swamp	22.80		22.80	
General Expenses:				
Administration Expense	16,429.92		16,429.92	
Endowment Fund Trustee and Safe-Keeping	1,033.70		1,033.70	
Bad Debts	352.83		352.83	
Special Repairs 1944 Hurricane Damage	2,012.50		2,012.50	
Cost of Living Bonus	240.00		240.00	
Gift to Naples Biological Station	100.00		100.00	
Special Repairs, Brick Laboratory	11,510.00		11,510.00	
Interest	312.50		312.50	
Reserve for Depreciation	25,314.41		25,314.41	
	$234,537.13	$227,104.26	$124,313.99	$116,881.12
Excess of Expense over Income carried to Current Surplus		7,432.87		7,432.87
		$234,537.13		$124,313.99

EXHIBIT C

Marine Biological Laboratory Current Surplus Account, Year Ended December 31, 1946

Balance, January 1, 1946			$196,337.90
Add:			
Transfer from Reserve Fund		$15,000.00	
Reserve for Depreciation Charged to Plant Funds		25,314.41	40,314.41
			$236,652.31
Deduct:			
Excess of Expense over Income for Year		$ 7,432.87	
Payments from Current Funds during Year for Plant Assets:			
Buildings	$ 2,691.13		
Equipment	8,324.14		
Library	10,409.75		
	$21,425.02		
Less Received for Plant Assets Sold	3,306.90	18,118.12	
Transfers to Reserve Fund:			
Portion of Dividends from General Biological Supply House	$ 2,500.00		
Dividends from Crane Co.	875.00		
Gansett Property Profits, 1945	464.18	3,839.18	
Retirement Fund:			
Payments to Pensioners	$ 3,460.00		
Less Retirement Fund Income	200.11		
	$ 3,259.89		
Loss on Sale of Interest in Real Estate	1,030.34	4,290.23	
Loss on Sale of Gansett Property in 1946		81.60	
Adjustment of Sundry Accounts-Receivable, etc., account of Prior Years		529.94	34,291.94
Balance, December 31, 1946			$202,360.37

Respectfully submitted,

Donald M. Brodie,
Treasurer

V. REPORT OF THE LIBRARIAN

1946

The sum of $13,500 appropriated to the library in 1946 was expended as follows: books, $546.47; serials, $2,747.01; binding, $618.39; express, $74.34; supplies, $431.05; salaries, $7,886.13 ($1,700 of this sum was contributed by the Woods Hole Oceanographic Institution); back sets, $417.04; insurance, $45.00; total, $12,765.43. The cash receipts of the library totalled $331.61: for microfilms, $195.03 ($78.45 expenses paid by the library and accounted above under "sup-

plies"); sale of duplicates, $135.65; sale of the "Serial List" Biological Bulletin supplement number, $0.93. This sum, $331.61, reverts to the laboratory and does not include rent payments for library readers which are collected by the main office. There were 38 library readers accommodated in the library during the summer of 1946, and practically all of these for the whole season, an improvement over 1945 when many of the 49 readers rented desks for short terms.

Of the Carnegie of New York Fund, $1,534.46 was expended for the completion of four back sets and for the partial completion of one other.

The sum appropriated by the Woods Hole Oceanographic Institution in 1946 for purchases was $800. A balance of $802.27 remaining from 1945 made an available total of $1,602.27. The expenditures amounted to $1,910.43, overrunning the account by the sum of $308.16. In addition to the above, the Woods Hole Oceanographic Institution contributed $1,700 (see above under salaries).

During 1946 the library received 1,065 current journals: 320 (19 new) by subscription to the Marine Biological Laboratory; 43 (15 new) to the Woods Hole Oceanographic Institution; exchanges with the "Biological Bulletin" 433 (13 new; 68 reinstated foreign) and 96 (21 new; 17 reinstated foreign) with the Woods Hole Oceanographic Institution; 165 as gifts to the former and 8 to the latter. The Library acquired 261 books: 81 by purchase of the Marine Biological Laboratory; 53 by purchase of the Woods Hole Oceanographic Institution; 13 gifts by the authors; 22 by the publishers; 48 by miscellaneous donors; 23 from the French Embassy Library, New York City; 1 from Dr. R. S. Lillie; 3 from Dr. Oliver S. Strong; 1 from Dr. Alfred C. Redfield; and 16 from Mrs. Priscilla B. Montgomery. There were 43 back sets of serial publications completed; 15 purchased by the Marine Biological Laboratory (4 with the Carnegie Fund); 7 by the Woods Hole Oceanographic Institution; 5 by exchange of the "Biological Bulletin"; 14 by exchange with the Woods Hole Oceanographic Institution publications; and 2 by gift and exchange of duplicate material. Partially completed sets were 73: purchased by the Marine Biological Laboratory, 10 (1 with the Carnegie Fund); purchased by the Woods Hole Oceanographic Institution, 10; by exchange with the "Biological Bulletin," 9; by exchange with the Woods Hole Oceanographic Institution publications, 11; by gift and exchange of duplicate material, 33.

The reprint additions to the library were 4,618: current of 1945, 841; current of 1946, 97; and of previous dates, 3,680. There were gifts of reprints from Dr. Alfred C. Redfield; Dr. E. G. Butler; and from Dr. L. C. Wyman.

At the end of the year 1946 the library contained 55,312 bound volumes and 142,292 reprints.

It is discouraging to report that no part of the German journals that have been lacking since 1940 have come through. We have heard, however, by letter of February 28, 1947, from the Otto Harrassowitz Company that there is "in preparation" a box of "stored material" that "will leave Berlin in summer via the Library of Congress Mission Berlin." We are hoping to discover whether this means some volumes of our standing order of the war years. If nothing comes to us in this direct way or through other of the Library of Congress "Mission Berlin" shipments I understand that a plan is already under way to reprint such lacking volumes provided a sufficient number of libraries are interested to assure the expense of the undertaking.

VI. REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory

Gentlemen:

I submit herewith the report of the fifty-ninth session of the Marine Biological Laboratory for the year 1946.

The past year has seen a continued recovery from war-time conditions. Our attendance increased, especially in the groups of Beginning Investigators and students, in large part due to returning veterans; many foreign journals have begun to come regularly after a lapse of several years; our gross income (and also expense) exceeded that of any recent year; the budget for research equipment was greatly increased; a good start was made in the repair of our buildings; the Lalor Fellowships were established.

1. Financial

During the past 10 years notable changes have occurred in the net income from some of our regular sources of revenue. Decreases appear in the return from the Endowments, from Instruction, and from the Mess; increases are seen in Research fees and the Supply Department. Changes in the cost of running the various departments have been due to war conditions. The accompanying table gives only the more important items of the budget. Net incomes are shown, rather than gross incomes. For the Supply Department, the Dormitories, and the Mess, the amounts do not include depreciation and other bookkeeping charges.

Net Income

	Endowments	Dividends	Research	Instruction	Supply Dept.	Dorm.	Mess
1946	35,965	18,270	14,315	(2,760)	14,545	5,290	780
1944	33,010	17,295	3,675	(2,915)	11,780	875	790
1942	36,085	11,420	4,950	(2,065)	1,760	9,275	1,385
1940	44,140	18,940	15,070	(615)	5,030	7,080	2,120
1936	50,190	12,700	10,070	2,130	9,160	6,660	3,125

Expense

	Bldgs. and Grds.	App. and Chem.	Library	Spec. Repairs	Admin.	Pensions
1946	37,420	15,055	17,075	13,525	16,430	3,460
1944	32,675	6,135	12,315	2,465	15,275	3,460
1942	25,130	9,805	15,335	5,810	12,500	3,460
1940	28,785	19,005	17,780	1,650	12,425	3,710
1936	37,925	17,190	22,140	—	14,910	4,060

Most important is the decline in the return from the Endowments. In 1930 our receipts from these sources amounted to $57,000. A sharp drop occurred in 1933, and smaller but steady declines each year until 1946. Our income now is less than two-thirds of the 1930 amount. In the meantime, returns from Dividends

have risen, and have made up nearly half of the loss incurred in the Endowment income. The budget for Instruction showed a substantial loss during the war years when attendance was light. With larger numbers of students and an increase in tuition from $75.00 to $100.00 the deficit should disappear. The net gain from the Mess has fallen materially. In view of the high cost of food and services we are fortunate to end each season without loss.

On the other hand, the net return from Research fees is now high, due in part to rentals received from the Oceanographic Institution which has occupied rooms throughout the year. The Supply Department has been exceedingly active. For 1946 the gross receipts were over $73,000, as compared with $43,000 ten years ago. However, the net income has not increased proportionately, for the cost of specimens purchased from dealers, of containers, chemicals, and other items, has kept down the profits. Salaries have been increased by more than 25 per cent.

The major categories of expense, shown on the lower part of the Table are now, with one exception, only a little above what they were in 1936. During the war we could not obtain many items of scientific equipment and so could not replace outworn apparatus. The budget for the Apparatus Department was, therefore, small. Now when equipment is available we can not buy all that we need because of its high cost. So, although we are spending as much as we did 10 years ago, we obtain far less. Our physical plant fell into disrepair, but is now gradually being put into sound condition. The cost of this work in 1946 is shown in the column Special Repairs.

We stand in need of additional funds to increase our endowments, to improve and enlarge our research facilities, to make further essential repairs in our buildings, to improve the working conditions of the Supply Department, and finally, to replace the wooden laboratories with a modern, well equipped building. The Executive Committee submitted to the Rockefeller Foundation a full statement of our needs with a request for funds to meet all or a part of them. The Foundation, in reply, recognized the importance of these projects but stated that because of its large contribution to the Peking Union Medical College, it could not at this time consider our request. In view of this, the Laboratory, should seek moderate contributions from a number of sources, thus broadening our base of support. One such source is our own membership which has been asked to contribute to a fund for a new collecting boat. The response has been encouraging, the donors expressing their hearty approval of the project.

2. Buildings and Grounds

All of the Laboratory buildings will be in active use during the summer of 1947. The Lecture Hall, which was stripped of its salt water and gas when the Navy occupied it, is now fully restored and will be filled with investigators. Rooms in the Supply Department will also be used for research.

Repairs and replacements have been made elsewhere. The sea wall is now rebuilt, the waterproofing of the Brick Building is finished. The experience of the past winter has proved that the operation was successful. The Dormitory is now undergoing the same treatment. In addition, many of its rooms, left unpainted since the building was erected more than 20 years ago, will be painted. A most desirable improvement is the partial painting of the Auditorium.

Room 106 in the Brick Building has been transformed into an Associates Room. The Trustees, at the Special Meeting in Boston, directed that this be done in response to the request of the Associates who want some place in the Laboratory where they can meet. These friends will constitute an increasingly important part of our membership.

A part of the Devil's Lane Tract is now open, and roads through it have been constructed. Thus far 8 lots have been purchased by members of the Corporation. Now that all lots in the Gansett tract are sold, this new area is the only Laboratory property which is available to investigators who want to solve the housing problem by owning their own homes.

3. The Lalor Fellowships

Through the generosity and cooperation of the Lalor Foundation this Laboratory, during the next five years, can offer to well prepared young investigators, a place to work, adequate equipment, and funds sufficient for their living and travelling expenses. The Fellows will work primarily in the fields of Biophysics, Biochemistry, and Physiological Chemistry, but other fields are not excluded. Dr. Metz who conducted the negotiations with Dr. Burdick of the Lalor Foundation is to be congratulated on their successful conclusion.

4. Gifts

The Laboratory acknowledges with sincere thanks gifts from the following donors:

The Associates: $1,070 for the purchase of special apparatus.

Mrs. Murray Crane: A $1,000 bond, the proceeds of which have been used to purchase special apparatus.

Dr. W. D. Curtis: $100 for carpenter shop equipment.

Dr. A. C. Redfield: $100 for trees and shrubs.

Mr. Laurence Saunders: $200 for the bathing beach raft.

5. Losses by Death

The Laboratory has lost by death, since the last meeting of the Trustees, five men, three of whom were closely associated with the institution for more than 50 years.

Prof. William B. Scott, member of the Corporation since 1890; elected Trustee in 1897; Emeritus, 1931.

Prof. Robert A. Harper, Corporation member since 1891; Trustee from 1911 until his retirement in 1926 when he became Emeritus.

Mr. George M. Gray, laboratory assistant in 1891; full time manager of the Supply Department from 1899 until his retirement in 1933.

Prof. Herbert S. Jennings, Trustee from 1905 to 1938, when he was elected Trustee Emeritus.

Prof. Samuel O. Mast, Trustee from 1936 to 1943; Trustee Emeritus until his death.

We honor the memory of these men who through many years greatly helped in the development of this Laboratory.

6. Election of Trustees

At the Meeting of the Corporation on August 13, 1946, the following trustees were elected:

Class of 1950

D. E. S. Brown
D. P. Costello
M. H. Jacobs
D. M. Marsland
A. K. Parpart
F. Schrader
H. B. Steinbach
B. H. Willier

Class of 1949

F. A. Brown, Jr., to replace W. C. Curtis retired

Trustee Emeritus

W. C. Curtis

7. There are appended as parts of this report

1. Memorials to Dr. T. H. Morgan and C. E. McClung.
2. The Staff.
3. Investigators and Students.
4. Tabular View of Attendance.
5. Subscribing and Cooperating Institutions.
6. Evening Lectures.
7. Shorter Scientific Papers (Seminars).
8. Members of the Corporation.

Respectfully submitted,

CHARLES PACKARD,
Director

1. MEMORIALS TO DECEASED TRUSTEES

Thomas Hunt Morgan, 1866–1945

by E. G. Conklin

In the death of T. H. Morgan at Pasadena, California, on December 4, 1945, the world of science lost one of its foremost students of Experimental Zoology and Genetics, and the Marine Biological Laboratory one of its earliest and most eminent members. He was born at Lexington, Kentucky, September 25, 1866, member of a family distinguished in American history for patriotic and military achievements. He graduated from the University of Kentucky in 1886 with the degree of B.S. and was given the M.S. Degree in 1887. In the summer of 1886 he was a student at the marine laboratory at Annisquam, Massachusetts, during the last session of that laboratory, which was the immediate predecessor of the Woods Hole Marine Biological Laboratory. Thus he was the last surviving personal link between the Annisquam and the Woods Hole Laboratories. The Annisquam Laboratory was not widely known, although it was the successor through its founder, Alpheus Hyatt, of

the famous but short-lived laboratory established by Louis Agassiz at Penikese Island in 1872. It would be interesting to learn how Morgan, immediately after his undergraduate course, came to attend this last session of the Annisquam Laboratory, but the fact of his attendance is evidence of his early interest in marine biology.

In the fall of 1886 he entered the Johns Hopkins University as a graduate student in the department of biology taking morphology under W. K. Brooks as his major subject and physiology under H. Newell Martin as his minor. His superior qualities were soon recognized by his appointment as teaching assistant during his first two years and by the University fellowship in biology during his third year. He received the Ph.D. degree in 1890 and was at once appointed to the Adam Bruce post-doctoral fellowship for the following year, part of which he spent on research expeditions in the Bahamas and Jamaica.

On his return from the West Indies in 1891 he was appointed Associate Professor of Biology at Bryn Mawr College, in succession to Professor E. B. Wilson who had been called to Columbia University. Morgan remained at Bryn Mawr as Associate Professor and Professor until 1904, when he was appointed Professor of Experimental Zoology at Columbia, where he remained for 24 years, during which his research work in genetics received world-wide recognition and, together with that of his students and associates, led to many fundamental discoveries regarding the mechanism of heredity and sex. Finally in 1928 he accepted the position of Professor of Biology and Director of the Wm. G. Kerckhoff Laboratories of Biology at the California Institute at Pasadena, where he remained for the balance of his life.

From his first acquaintance with marine biology at Annisquam to the end of his life Morgan was closely associated with marine laboratories, especially during his summer vacations. In 1888 and 1889, while still a graduate student, he occupied the Johns Hopkins Table at the laboratory of the U. S. Fish Commission at Woods Hole. Those were the first two years of the M. B. L. and although he is not listed as a member of this new laboratory for those years, he was closely associated with it from its beginning, for relations between these two Woods Hole laboratories in those days of few workers were very intimate. In 1890 Morgan became an active member of the M. B. L., occupied a room in the laboratory, gave lectures in the Zoology course, and one of the evening lectures, and became a member of the Corporation. In 1899 he became a life member, in 1897 a trustee and in 1936 trustee emeritus. During this long official connection, extending over 55 years, he was always an active collaborator in the plans and work of the laboratory, and was one of its most productive and inspiring investigators. He was for many years a member of the Executive Committee as well as of other special committees and his judgment and cooperation had much to do in shaping policy. Although he believed that once the laboratory was well endowed it should abandon teaching and devote all its resources to research, he nevertheless cooperated in the past and present policy of combining both teaching and research. In his earlier years here he lectured in some of the courses, and throughout his connection with the laboratory he served as a member of the "Staff of Investigation" and supervised the work of many research students. His general lectures to the whole laboratory were many and notable summaries of his research work. Sixteen such "Evening Lectures" are listed in the Annual Reports of the Laboratory and many of these were published in the former series of *Biological Lectures* or in the later *Biological Bulletin*.

He was rarely absent from the sessions of this laboratory, except in 1894, 1895 and 1900 when he was at the Zoological Station in Naples. Even after his chief interests had turned from experiments on marine animals to the genetics of insects he continued to come to Woods Hole summers, transporting his material and continuing his work here. And after his removal to California he and Mrs. Morgan continued to make the long journey to Woods Hole every summer until prevented by war conditions in the past two years. In California he made frequent visits to the marine laboratory of the California Institute at Corona del Mar, which he had established, and his last work there was done last summer on "Normal and Abnormal Development of the Eggs of Ciona" and was published about a month after his death.

When he was a student at Johns Hopkins, morphology, which was his chief subject, consisted largely of observational rather than experimental work and was centered to a large extent on the embryology of various classes of animals with the primary purpose of determining their phylogeny and relationships. Morgan's early researches were of this sort. By the time he had taken his Ph.D. he had finished eight papers and in the two following years he published eight more on this general theme. After 1892 he published no papers that were not experimental or analytical. This sudden change in his interests was due in large part to associations formed and interests aroused at Woods Hole and at the Naples Zoological Station. His experiments at Woods Hole began on teleost and echinoderm eggs in 1893, and at Naples in 1894 he collaborated with Hans Driesch on the embryolgoy of ctenophores and began experiments on many other groups. Thereafter he was always interested in the factors controlling development and always had some work going on that subject even when he was more deeply involved in other projects. In all he published about sixty papers and three books on the experimental embryology of animals belonging to ten different phyla or classes. Another field in which he did an enormous amount of work is regeneration, on which he published about forty papers and two books, on some eight classes of animals.

About the time of his removal to Columbia University in 1904 he began work on general cytology and especially on the cytology of sex determination. This led to extensive studies on the Cytological basis of peculiar sex ratios in parthenogenesis and on non-typical Mendelian ratios in general. Here also may be classed his studies on sex-limited and sex-linked heredity together with the causes of secondary sexual characters, as well as his numerous papers on fertility and sterility, which continued intermittently until the end of his life. In this field he published some fifty papers and two books.

Finally in the general field of Mendelism, mutations, and the "constitution of the germ plasm," he published either individually or in collaboration with assistants, about one hundred and twenty-five papers and ten books. Other publications of a more general character, such as those on evolution, natural selection, reviews, and biographies, bring the total number of his publications, as listed in the bound volumes of his reprints in our library and in unbound separates since 1937, to three hundred and seventy separate titles, of which twenty-four are books, some of which are translations into the Dutch, German, Spanish and Russian languages.

This long list of Morgan's published work on a great variety of subjects throws light on many of his personal characteristics. His biological interests were very extensive and he was extraordinarily alert in picking up new leads in research. He worked with great intensity and rapidity and published his journal articles promptly,

and sometimes without adequate caution; as a result he often made mistakes. His laboratory room was always open to callers, but unless their business was important he did not permit them to interrupt his work. He would go right on with what he was doing and would answer questions without looking up from his microscope or dishes. But he was always cordial and helpful and if the business were important he would give it prompt attention. In more leisurely moments he loved to joke and gossip and was gay and full of fun, but in the laboratory he usually kept right on with his work. On one occasion during the first World War I wandered into his room and said, "This War is getting terrible." Without looking up he replied, "What war?" And yet he was as anxious as anyone about the course of events. He dwelt in no "ivory tower," but just then he was most interested in counting flies. I think I have never known any other man who wasted so little time. His work was his life.

He could do good work with the most primitive accommodations and instruments, and he cared little for show and finesse. His work room and table and aquaria might be crowded and apparently disorderly but he usually knew just where to put his hand on what he wanted. His microscope slides and bottles and dishes might be crudely labelled but they served his purpose. In short, he wasted no time on needless techniques but went as directly as possible to the results which he sought.

He disliked organizational and committee work, which is often a great waste of time, but in really important matters he took an active and important part, as in the affairs of the Marine Biological Laboratory, the American support of the Naples Station, the founding of the Journal of Experimental Zoology, and many other institutional and professional matters. In a halting and confused situation no one could be more helpful than he, for with lightening-like rapidity he would isolate the main issues from minor ones and go straight to a logical conclusion. His judgment of men was especially sound and forthright and he did not allow it to be warped by personal sentiments. But he was genuinely kind and affectionate with family, friends, students and associates, and no one learned, except by chance, of his many kindnesses and contributions to persons and institutions. I forbear to say more on this subject out of respect to his well known wishes.

He received abundant recognition and honors from many universities and scientific societies in America and foreign countries; he was president of our leading biological and zoological societies as well as of the more inclusive American Association for the Advancement of Science and the more exclusive National Academy of Sciences; he was a foreign member of the Royal Society and the one and only American zoologist to receive the Nobel Award; but these honors never changed in any respect his sincere and modest behavior.

When he began his epoch-making work on *Drosophila* who could have predicted the amazing discoveries which would be revealed in the course of a few years! It had been customary among students of heredity, following Weismann, to speak of "the architecture of the germ plasm," but practically nothing was known of that architecture except its location in the germ cells. After the work of Morgan and his associates that architecture became a detailed plan with precise location of hereditary factors or genes in chromosome maps, and in turn these maps led to fundamental discoveries in the phenomena of heredity itself. In this work he had the invaluable assistance of students who became his assistants and associates, and his judgment of men was never shown better than in his selection of these associates. Together they made an extraordinary team, working in harmonious cooperation and

in a spirit of great adventure. Morgan was the leader of this team and he received chief recognition and honor for their important discoveries but he always gave full credit to his associates and shared his honors with them. And now this great leader and discoverer is gone from among us but his work and inspiration will remain with us and with future generations.

Professor C. E. McClung

April 5, 1870 to January 17, 1946

by F. R. Lillie

It is difficult, and indeed impossible in a short space, to do justice to the many-sided life of McClung. Here we record our sorrow and sense of loss to our own institution and express our gratitude for the many services that he rendered us. A graduate in Pharmacology of the University of Kansas in 1892, he received his A.B. Degree there in 1896 and, after study elsewhere, his Ph.D. Degree in 1902. He served as head of the department of Zoology at the University of Kansas until he succeeded the lamented Thomas H. Montgomery at the University of Pennsylvania in 1912. He continued unbroken Montgomery's loyalty and devotion to the M. B. L. and was elected a member of the Corporation and Trustee in 1913. With the exception of the year 1934 when he was visiting professor at Keio University in Tokyo, his membership was continuous until his death.

His first appearance at this Laboratory was as investigator and instructor in Embryology in 1903. Thereafter he was occupied in the work of the University of Kansas until his appointment at the University of Pennsylvania. He was then appointed on the M. B. L. staff of investigations and served throughout his life assisting not only his own students but many others who sought his advice. He also became interested in the library and served as Chairman of the Library Committee from 1923 to 1933 during the great development of the library after the erection of the main building in 1924; he was especially helpful when the General Education Board in 1925 presented $50,000.00 to be expended over a period of five years for purchase of books, periodicals and pamphlets, and in 1929 $200,000.00 for endowment of the library.

After the erection of the Crane Laboratory in 1913, the Laboratory rapidly became over-crowded again, and in 1919 the trustees decided that steps should be taken to provide construction and endowment that should be adequate to meet the requirements of a generation of biologists. One of the first steps toward this program was to approach the National Research Council through Dr. McClung, Chairman of the Division of Biology and Agriculture. He was instrumental in the appointment of a committee of his division to report on the situation. Their favorable report was approved by the Executive Board of the Council. This encouraged the presentation of the matter to the Rockefeller Foundation, and the raising of the funds for the great plan was soon well under way. Dr. McClung was also helpful at the laboratory from the start of the new plans. The arrangement of the large lecture room, which has proved so successful, was his suggestion.

Many of us remember Dr. McClung's kindliness, simplicity of character and friendliness. These qualities were displayed in many way in the social life of Woods Hole; thus, he served as President of the M. B. L. Tennis Club and also of the

M. B. L. Club, the social club of the students and investigators. Such activities, however, never detracted from his main interests in research, and a significant part of his most famous investigations on chromosomes was carried on here.

He was a member of the National Academy of Sciences, of the American Philosophical Society and of numerous national biological societies, and in most of them occupied positions of trust and influence at various times. He was indeed a consecrated man of science! His memory will long endure.

2. The Staff, 1946

Charles Packard, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
Ralph S. Lillie, Professor of General Physiology, Emeritus, The University of Chicago.
S. O. Mast, Professor of Zoology, Emeritus, Johns Hopkins University.
A. P. Mathews, Professor of Biochemistry, Emeritus, University of Cincinnati.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.

ZOOLOGY

I. Consultants

T. H. Bissonnette, Professor of Biology, Trinity College.
L. L. Woodruff, Professor of Protozoology, Yale University.

II. Instructors

F. A. Brown, Associate Professor of Zoology, Northwestern University, in charge of course.
T. H. Bullock, Assistant Professor of Neurology University of Missouri Medical School.
W. D. Burbanck, Associate Professor of Biology, Drury College.
C. G. Goodchild, Professor of Biology, Southwest Missouri State Teachers College.
John H. Lochhead, Instructor in Zoology, University of Vermont.
Madelene E. Pierce, Assistant Professor of Zoology, Vassar College.
W. M. Reid, Assistant Professor of Biology, Monmouth College.
Mary D. Rogick, Professor of Biology, College of New Rochelle.

III. Laboratory Assistant

Antoin Baca, Duke University Medical School.

EMBRYOLOGY

I. Consultants

H. B. Goodrich, Professor of Biology, Wesleyan University.

II. Instructors

Donald P. Costello, Professor of Zoology, University of North Carolina, in charge of course.
Howard L. Hamilton, Assistant Professor of Zoology, Iowa State College.

JOHN A. MOORE, Assistant Professor of Zoology, Barnard College.
ALBERT TYLER, Assistant Professor of Embryology, California Institute of Technology.

III. RESEARCH ASSISTANT

MARJORIE HOPKINS FOX, University of California.

IV. LABORATORY ASSISTANTS

CATHERINE HENLEY, The Johns Hopkins University.
ELEANOR LERNER, Washington University.

PHYSIOLOGY

I. CONSULTANTS

WILLIAM R. AMBERSON, Professor of Physiology, University of Maryland, School of Medicine.
HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.
WALTER E. GARREY, Professor of Physiology, Vanderbilt University Medical School.
MERKEL H. JACOBS, Professor of Physiology, University of Pennsylvania.

II. INSTRUCTORS

ARTHUR K. PARPART, Associate Professor of Biology, Princeton Universtiy, in charge of course.
E. S. GUZMAN BARRON, Associate Professor of Biochemistry, The University of Chicago.
EDWARD CHAMBERS, New York City.
AURIN M. CHASE, Assistant Professor of Biology, Princeton University.
ARTHUR C. GIESE, Associate Professor of Biology, Stanford University.
RUDOLPH T. KEMPTON, Professor of Zoology, Vasser College (absent in 1946).
ROBERT RAMSEY, Associate Professor of Physiology, Medical College of Virginia.

BOTANY

I. CONSULTANTS

S. C. BROOKS, Professor of Zoology, University of California.

II. INSTRUCTORS

MAXWELL S. DOTY, Instructor of Botany, Northwestern University.
HANNAH CROASDALE, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. FAILLA, College of Physicians and Surgeons, Columbia University.
L. ROBINSON HYDE, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

PRISCILLA B. MONTGOMERY (MRS. THOMAS H. MONTGOMERY, JR.), Librarian
DEBORAH LAWRENCE, Assistant Librarian
MRS. ELON JESSUP MARY A. ROHAN

APPARATUS DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager
J. D. Graham — Dorothy LeFevre

CHEMICAL DEPARTMENT

E. P. Little, Phillips Exeter Academy, Exeter, N. H., Manager

SUPPLY DEPARTMENT

James McInnis, Manager
D. J. Zinn, Naturalist

Ruth Crowell — Grace M. Witzell
M. B. Gray — W. E. Kahler — F. N. Whitman
A. M. Hilton — G. Lehy

GENERAL OFFICE

F. M. MacNaught, Business Manager
Polly L. Crowell — Mrs. Lila S. Myers

GENERAL MAINTENANCE

T. E. Larkin, Superintendent
W. C. Hemenway — G. T. Nickelson, Jr.
R. W. Kahler — T. E. Tawell
A. J. Pierce

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus

3. Investigators and Students

Independent Investigators, 1946

Abell, Richard G., Assistant Professor of Anatomy, University of Pennsylvania.
Addison, William H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
Anderson, Rubert S., Assistant Professor of Physiology, University of Maryland.
Anfinsen, Christian B., Instructor in Biological Chemistry, Harvard Medical School.
Armstrong, Philip B., Professor of Anatomy, College of Medicine, Syracuse University.
Ball, Eric G., Associate Professor of Biological Chemistry, Harvard Medical School.
Barron, E. S. Guzman, Associate Professor of Biochemistry, University of Chicago.
Bartlett, James H., Professor of Theoretical Physics, University of Illinois.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bevelander, Gerrit, Associate Professor of Anatomy, New York University.
Bliss, Alfred F., Instructor in Physiology and Pharmacology, Albany Medical College.
Boell, Edgar J., Associate Professor of Biology, Yale University.
Bonner, John T., Junior Fellow, Harvard University.
Bridgman, Josephine, Associate Professor of Biology, Limestone College.
Brink, Frank, Jr., Research Associate, Johnson Foundation, University of Pennsylvania.
Brooks, Matilda M., Research Associate in Biology, University of California.
Brooks, Sumner C., Professor of Zoology, University of California.
Brown, Dugald E. S., Professor of Physiology, New York University.
Brown, Frank A., Jr., Associate Professor of Zoology, Northwestern University.

Brownell, Katherine A., Research Associate, Ohio State University.
Buck, John, National Institute of Health, Bethesda, Maryland.
Budington, Robert A., Professor of Zoology, Emeritus, Oberlin College.
Bullock, Theodore H., Assistant Professor of Anatomy, University of Missouri.
Burbanck, William D., Professor of Biology, Drury College.
Burkholder, Paul R., Professor of Botany, Yale University.
Cannan, R. Keith, Professor of Chemistry, New York University.
Chambers, Robert, Research Professor of Biology, New York University.
Chase, Aurin M., Assistant Professor of Biology, Princeton University.
Cheney, Ralph Holt, Chairman of Biology Department, Long Island University.
Chrystall, Frieda L., Teacher of Biology, Julia Richman High School.
Claff, C. Lloyd, Research Fellow in Surgery, Harvard Medical School.
Clark, Eleanor Linton, Voluntary Research Worker, University of Pennsylvania.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania School of Medicine.
Clement, A. C., Associate Professor of Biology, College of Charleston.
Clowes, G. H. A., Research Director, Emeritus, Eli Lilly and Company.
Cole, Kenneth S., Professor of Biophysics, University of Chicago.
Conklin, Edwin G., Professor of Biology, Emeritus, Princeton University.
Cooper, Kenneth W., Associate Professor of Biology, Princeton University.
Copeland, D. Eugene, Assistant Professor of Zoology, Brown University.
Copley, Alfred E., Research Associate, Mount Sinai Hospital.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Croasdale, Hannah T., Technical Assistant, Dartmouth College.
Crouse, Helen V., Research Associate in Zoology, University of Pennsylvania.
Crowell, Sears, Associate Professor of Zoology, Miami University.
Curtis, W. C., Professor of Zoology, University of Missouri.
Dalcq, Albert M., Head of Anatomy and Embryology, Faculty of Medicine, Brussels.
Dalton, Howard C., Instructor in Biology, Brown University.
Dean, Helen W., Department of Anatomy, Harvard Medical School.
Diller, Irene Corey, Research Cytologist, Institute for Cancer Research, Lankinau Hospital.
Doty, Maxwell S., Instructor in Botany, Northwestern University.
Dumm, Mary E., Instructor in Chemistry, New York University Medical College.
Ellinger, Tage U. H., Professor of Zoology, Howard University.
Erdman, J. Gordon, Fellow, Mellon Institute of Industrial Research.
Failla, G., Professor of Radiology, Columbia University.
Figge, Frank H. J., Associate Professor of Anatomy, University of Maryland School of Medicine.
Fiske, Virginia Mayo, Instructor in Zoology, Wellesley College.
Frisch, John A., Professor of Biology, Canisius College.
Froehlich, Alfred, Associate, May Institute for Medical Research.
Furth, Jacob, Professor of Pathology, Cornell University Medical College.
Garrey, Walter E., Professor of Physiology, Emeritus, Vanderbilt University.
Giese, Arthur C., Associate Professor of Biology, Stanford University.
Gilbert, Perry W., Assistant Professor of Zoology, Cornell University.
Gilman, Lauren C., Assistant Professor of Zoology, University of South Dakota.
Glaser, Otto C., Professor of Biology, Amherst College.
Goodchild, Chauncey G., Professor of Biology, Missouri State College.
Gould, Harley N., Professor of Biology, H. Sophie Newcomb College.
Grand, C. G., Research Associate, New York University.
Gray, I. E., Professor of Zoology, Duke University.
Gregg, John R., Instructor in Biology, Johns Hopkins University.
Grundfest, Dr. Harry, Research Associate, Department of Neurology, Columbia University.
Harding, Clifford V., Assistant in Physiology, Brown University.
Harnly, Morris H., Associate Professor, Washington Square College.
Hartman, Frank A., Professor and Chairman, Department of Physiology, Ohio State University.
Harvey, Ethel Browne, Independent Investigator, Princeton University.
Harvey, E. Newton, Professor of Physiology, Princeton University.

HAYWOOD, CHARLOTTE, Professor of Physiology, Mount Holyoke College.
HEILBRUNN, L. V., Professor of Zoology, University of Pennsylvania.
HIBBARD, HOPE, Professor of Zoology, Oberlin College.
HOPKINS, HOYT S., Associate Professor of Physiology, New York University.
HUNTER, FRANCIS ROBERT, Assistant Professor of Zoology, University of Oklahoma..
HUTCHINGS, LOIS M., Instructor in Biology, New York University.
IFFT, JOHN D., Assistant Professor of Biology, Simmons College.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JAKUS, MARIE A., Research Associate in Biology, Massachusetts Institute of Technology.
JENKINS, GEORGE B., Professor of Anatomy, Emeritus, George Washington University.
JEROME, SISTER FRANCIS, Instructor, Ohio State University.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt Medical School.
KAAN, HELEN W., Associate Professor of Zoology, Wellesley College.
KEMP, MARGARET, Assistant Professor of Botany, Smith College.
KEMP, NORMAN E., Instructor in Biology, Wayne University.
KINDRED, JAMES E., Professor of Anatomy, University of Virginia.
KLEINHOLZ, LEWIS H., Guggenheim Fellow, Harvard University.
KLOTZ, JOHN W., Instructor in Biology, Concordia Teachers College.
KREEZER, GEORGE L., Guggenheim Fellow, Princeton University.
KRUGELIS, EDITH J., Instructor of Zoology, Vassar College.
KUNTZ, ELOISE, Assistant in Biology Department, Brown University.
LAVIN, GEORGE I., In charge of Spectroscopic Laboratory, Rockefeller Institute for Medical Research.
LAZAROW, ARNOLD, Instructor in Anatomy, Western Reserve Medical School.
LEFEVRE, PAUL G., Instructor in Physiology, University of Vermont, College of Medicine.
LILLIE, RALPH S., Professor of Physiology, Emeritus, The University of Chicago.
LILLY, DANIEL M., Assistant Professor in Biology, Providence College.
LLANA, DR. ALFRED H., Assistant Director, University of Chile.
LOCHHEAD, JOHN H., Assistant Professor of Zoology, University of Vermont.
LUCKE, BALDUIN, Professor of Pathology, University of Pennsylvania School of Medicine.
LYNCH, REV. WM. F., Teaching Fellow, New York University.
MANGINELLI, PIERO, Assistant, Faculdade de Medicina, Sao Paulo, Brazil.
MARINELLI, LEONIDES D., Physicist, Memorial Hospital.
MARMONT, GEORGE H., Assistant Professor of Biophysics, University of Chicago.
MARSLAND, DOUGLAS, Associate Professor of Biology, New York University.
MAST, S. O., Professor of Zoology, Emeritus, Johns Hopkins University.
MATHEWS, ALBERT P., Professor of Biochemistry, Emeritus, University of Cincinnati.
MENDES, ERASMO GARCIA, Assistant Professor, University of Sao Paulo, Brazil.
MENKIN, VALY, Assistant Professor of Pathology, Duke University School of Medicine.
METZ, CHARLES W., Director, Zoological Laboratory, University of Pennsylvania.
MOORE, JOHN A., Assistant Professor of Zoology, Barnard College.
NACHMANSOHN, DAVID, Research Associate in Neurology, Columbia University.
NELSON, LEONARD, Independent Investigator, University of Pennsylvania.
NORTHROP, JOHN H., Member of the Institute, Rockefeller Institute for Medical Research.
OPPENHEIMER, JANE M., Assistant Professor of Biology, Bryn Mawr College.
ORMSBEE, RICHARD A., Associate, Sloan-Kettering Institute, Memorial Hospital.
ORR, PAUL R., Assistant Professor, Brooklyn College.
OSTER, ROBERT H., Professor of Physiology, University of Maryland School of Medicine.
OSTERHOUT, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
PALADE, GEORGE, Assistant Department of Anatomy, University of Bucharest, Rumania.
PARPART, ARTHUR K., Professor of Biology, Princeton University.
PIERCE, MADELENE E., Associate Professor of Zoology, Vassar College.
PIERSON, BERNICE F., Independent Investigator, Johns Hopkins University.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
POULSON, D. F., Assistant Professor of Biology, Yale University.
RAKESTRAW, NORRIS W., Research Associate, Woods Hole Oceanographic Institute.
RAMSEY, ROBERT W., Associate Professor of Physiology, Medical College of Virginia.
RANKIN, JOHN S., Assistant Professor of Zoology, University of Connecticut.

Reid, W. Malcolm, Head of Biology Department, Monmouth College.
Reinhard, Edward G., Professor of Biology and Head of Department, Catholic University of America.
Ris, Hans, Assistant in Physiology, Rockefeller Institute.
Robbie, Wilbur A., Research Associate, State University of Iowa.
Rogick, Mary Dora, Professor of Biology, College of New Rochelle.
Ryan, Francis, Assistant Professor of Zoology, Columbia University.
Schaeffer, A. A., Chairman Department of Biology, Temple University.
Scharrer, Ernst, Associate Professor of Anatomy, University of Colorado School of Medicine.
Schmidt, Gerhard, Senior Research Fellow in Medicine, Tufts College Medical School.
Schneyer, Leon H., Instructor, New York University Dental College.
Scott, Sister Florence Marie, Professor of Biology, Seton Hill College.
Shanes, Abraham M., Assistant Professor of Physiology, New York University College of Dentistry.
Shereshefsky, J. Leon, Professor of Physical Chemistry, Howard University.
Sichel, Dr. F., Professor of Physiology, University of Vermont College of Medicine.
Slifer, Eleanor H., Assistant Professor of Zoology, State University of Iowa.
Steinbach, H. B., Associate Professor of Zoology, Washington University.
Stewart, Dorothy R., Research Associate in Physiology, University of Pennsylvania Medical School.
Stokey, Alma G., Professor of Botany, Emeritus, Mount Holyoke College.
Stunkard, Horace H., Professor of Biology, New York University.
Sturtevant, Alfred H., Professor of Genetics, California Institute of Technology.
Taft, Charles H., Associate Professor of Pharmacology, University of Texas.
Taylor, Wm. Randolph, Professor of Botany, University of Michigan.
TeWinkel, Lois E., Associate Professor of Zoology, Smith College.
Thompson, Betty F., Instructor in Botany, Connecticut College for Women.
Todd, Edgar W., Visiting Investigator, Rockefeller Institute.
Tracy, Henry C., Professor of Anatomy, University of Kansas.
Tyler, Albert, Assistant Professor of Embryology, California Institute of Technology.
Villee, Claude A., Instructor in Biological Chemistry, Harvard Medical School.
Wainio, Walter W., Assistant Professor, New York University College of Dentistry.
Warner, Robert C., Assistant Professor of Chemistry, New York University College of Medicine.
Weber, Neal A., Associate Professor of Anatomy, University of North Dakota.
Weiss, Dr. Paul, Professor of Zoology, University of Chicago.
Wenrich, D. H., Professor of Zoology, University of Pennsylvania.
Whiting, Anna R., Instructor in Zoology, University of Pennsylvania.
Whiting, P. W., Associate Professor of Zoology, University of Pennsylvania.
Wichterman, Ralph, Assistant Professor of Biology, Temple University.
Wiercinski, Floyd J., Chairman and Professor of Biology, Lewis College of Science and Technology.
Wilber, Charles G., Instructor in Physiology, Fordham University.
Willier, B. H., Professor of Zoology, Johns Hopkins University.
Woodward, Arthur A., Jr., Research Assistant in Zoology, University of Pennsylvania.
Wrinch, Dorothy, Lecturer, Smith College.
Yntema, Chester L., Assistant Professor of Anatomy, Cornell University Medical College.

Beginning Investigators, 1946

Bender, Albert, Investigator, University of Pennsylvania.
Benedict, Dora, Student, Harvard Medical School.
Berg, George G., Graduate Student, Columbia University.
Blumenthal, Gertrude, University of Pennsylvania.
Brust, Manfred, Teaching Fellow in Biology, Washington Square College.
Chambers, Edward L., Research Associate, University of California.
Douglis, Marjorie B., Assistant in Zoology Department, University of Chicago.
Fisk, Albert A., Brown Fellow in Pathology, Yale University School of Medicine.

GOREAU, THOMAS F., Student, University of Pennsylvania School of Medicine.
GOULD, DAVID, Voluntary Investigator, New York University College of Medicine.
GREEN, JAMES W., Graduate Student, Princeton University.
GREENBERG, RUVEN, Graduate Student, Ohio State University.
GREGORY, JOHN C., Graduate Student, Yale University.
KOZAM, GEORGE, Graduate Student and Research Assistant, New York University Graduate School.
LEIN, JOSEPH, Graduate Student, Princeton University.
LOVELACE, ROBERTA, Teaching Fellow, University of North Carolina.
MCLEAN, DOROTHY J., Demonstrator, University of Toronto.
MOUL, EDWIN T., Teaching Fellow, University of Pennsylvania.
NARAHARA, HIROMICHI, Columbia College of Physicians and Surgeons.
RECKNAGEL, RICHARD O., Graduate Student, University of Pennsylvania.
REMOND, A., Research Fellow, Johnson Foundation, University of Pennsylvania.
ROGER, HENRY C., Student, Deerfield Academy.
ROYS, CHESTER C., University of Chicago.
SHENG, T. C., Graduate Student, Columbia University.
TAYLOR, BABETTE, Graduate Student, Washington University.
WEINER, MILTON H., Student, Western Reserve University.
WEISS, MICHAEL S., Teaching Fellow in Biology, New York University.
WHITE, ELIZABETH L., Graduate Student, Bryn Mawr College.
WILSON, T. HASTINGS, University of Pennsylvania Medical School.
WILSON, WALTER L., Graduate Student, University of Pennsylvania.

Research Assistants, 1946

ABRAMSKY, TESS A., Research Assistant, Rockefeller Institute for Medical Research.
BERMAN, MARJORIE, Research Assistant, Columbia University.
BURT, AGNES S., Research Assistant, University of Chicago.
CAMPBELL, MARGARET L., Research Assistant, California Institute of Technology.
CARLSON, FRANCIS D., Research Assistant, Johnson Foundation, University of Pennsylvania.
DEFALCO, ROSE H., Research Assistant, University of Pennsylvania.
DEY, THOMAS E., Research Assistant, Princeton University.
DIETZ, ALMA, Research Assistant, American International College.
FOX, MARJORIE H., Research Fellow, University of California.
HOPKINS, C. NADINE, Graduate Student, Northwestern University.
KERSCHNER, JEAN, Research Assistant, University of Pennsylvania.
LERNER, ELEANOR, Fellow in Zoology, Washington University.
MITCHELL, CONSTANCE, Research Assistant, University of Pennsylvania.
MITCHELL, ROBERT F., Research Assistant, New York University.
NORRIS, KARL H., Electronic Engineer, University of Chicago.
RALL, WILFRID, Graduate Fellow in Biophysics, University of Chicago.
ROBBIE, JEAN, Technical Assistant, Ohio State University.
ROSEN, MRS. DAVID, Research Assistant, Yale University.
ROTHENBERG, MORTIMER A., Research Assistant, College of Physicians and Surgeons, New York University.
SERIN, B. GOLDIS, Research Assistant, University of Pennsylvania.
ULLED, ELIAS, Research Assistant, Temple University Medical School.
VISHNIAC, WOLF, Research Assistant, Washington University.
WHEELER, CHARLES B., Part time Instructor, University of Kansas.
WILLIS, MARIAN, Research Assistant, University of Pennsylvania.
ZIEGLER, BERNICE, Laboratory Assistant, Washington University.

Library Readers, 1946

AMBERSON, WILLIAM R., Professor of Physiology, University of Maryland School of Medicine.
BAKER, GLADYS E., Associate Professor of Plant Science, Vassar College.
BENNIGHOF, C. L., Assistant Professor, Western Maryland College.

BLOCH, ROBERT, Research Associate in Botany, Yale University.
BLUM, HAROLD F., Guggenheim Fellow.
BOCHER, CAROL A., Research Assistant, The Johns Hopkins University.
CASSIDY, HAROLD G., Assistant Professor of Chemistry, Yale University.
COX, EDWARD H., Professor of Chemistry, Swarthmore College.
DISCHE, ZACHARIAS, Visiting Scholar, College of Physicians and Surgeons, New York University.
FRIEDEMANN, ULRICH, Head of Department of Bacteriology, Jewish Hospital, Brooklyn.
FRIES, E. F. B., Assistant Professor of Biology, City College of New York.
GATES, R. R., Professor of Botany Emeritus, University of London.
GRANT, MADELINE P., Member Science Faculty, Sarah Lawrence College.
GUDERNATSCH, FREDERICK, Visiting Professor, New York University.
GUREWICH, VLADIMIR, Assistant Visiting Physician, Bellevue Hospital.
HAMILTON, HOWARD L., Assistant Professor of Zoology, Iowa State College.
KELLER, RUDOLPH, Researcher, The Robinson Foundation, Inc.
KRASNOW, FRANCES, Head of Department of Research, Guggenheim Dental Foundation.
LOEWI, OTTO, Research Professor of Pharmacology, New York University College of Medicine.
LOW, EVA M., Graduate Student, Yale University.
LOWENSTEIN, OTTO, Clinical Professor of Neurology, New York University College of Medicine.
MCDONALD, SISTER ELIZABETH SETON, Professor of Biology, College of Mt. Saint Joseph-on-the-Ohio.
MAYOR, JAMES WATT, Research Professor of Biology, Union College.
MEYERHOF, OTTO, Research Professor of Biochemistry, University of Pennsylvania.
MORRIS, SAMUEL
MOSCHCOWITZ, ELI, Assistant Professor Chemical Medicine, Columbia University.
PONDER, ERIC, Research Investigator, The Nassau Hospital.
PRICE, WINSTON H., Research Assistant, Washington University Medical School.
SANDOW, ALEXANDER, Assistant Professor of Biology, New York University, Washington Square College.
SAVAGE, LEONARD J., Special Rockefeller Fellow, University of Chicago.
SCHRANK, A. R., Assistant Professor of Physiology, University of Texas.
SHWARTZMAN, GREGORY, Head of Department of Bacteriology, Mt. Sinai Hospital.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STERN, KURT G., Adjunct Professor of Biochemistry, Polytechnic Institute of Brooklyn.
STRAUS, WILLIAM L., JR., Associate Professor of Anatomy, Johns Hopkins University.
WHITE, MICHAEL J. D., Reader in Zoology, University College, London.
WINSOR, CHARLES P., Assistant Professor of Biology, Johns Hopkins School of Hygiene.
ZORZOLI, ANITA, Instructor in Physiology, Washington University School of Dentistry.

Students, 1946

BOTANY

CLIFFORD, SISTER ADELE, S. C., Fordham University.
FAHEY, ELIZABETH MARY, Student, Boston University.
FRAAS, JANET LOIS, Student, Wheaton College.
GARNIC, JUSTINE, Student, Carnegie Institute of Technology.
KEEFFE, MARY M., Student, Fordham University.
TORREY, ANNA MARSH, Student, Swarthmore College.
URQUHART, BETTY A., Instructor in Botany, Wheaton College, Wheaton, Illinois.
VISHNIAC, WOLF, Research Assistant, Washington University.
WILSON, MARIE ELLEN, Student, Western Maryland College.

EMBRYOLOGY

BANNER, ALBERT H., Assistant Professor, University of Hawaii.
BARISH, NATALIE, Student, Goucher College.

BRINSLEY, BERTRAM, Graduate Student, New York University.
BRUCH, PAUL ROBERT, Student, Wesleyan University.
BUCKLIN, DONALD HARTWELL, 124 Woodbine Street, Providence, Rhode Island.
DIDEA, ARTHUR, Assistant in Zoology, Washington University.
EBERT, JAMES DAVID, Student, Johns Hopkins University.
EISENBERG, NORMA, Graduate Student, Brooklyn College.
FITCH, NAOMI SELMA, Student, McGill University.
FLOOD, REV. FRANCIS X., S. J., Assistant Professor of Biology, Canisius College.
FRIEDMAN, FLORENCE L., Brooklyn College.
GALBREATH, JEAN L., Student Assistant, Elmira College.
HARRISON, JOHN W., Graduate Student, Washington and Jefferson College.
JONES, ALBERTA S., Student, Howard University.
KUFF, EDWARD LOUIS, Student, Johns Hopkins Medical School.
LEIGH, WALTER HENRY, Instructor, Chicago City Junior College.
LIU, CHIEN-KANG, McGill University.
MARKERT, CLEMENT LAWRENCE, Graduate Assistant, Johns Hopkins University.
MOORE, ELLEN LOUISE, Student, Wellesley College.
MORRIS, DAVID MARKLAND, JR., Graduate Assistant and Student, Indiana University.
MULLALLY, REV. WALTER BERNARD, Instructor in Biology, St. Anselm's College.
MURTLAND, RICHARD LEE, Graduate Student, Washington and Jefferson College.
PEQUEGNAT, WILLIS EUGENE, Assistant Professor of Zoology, Pomona College.
SCHECTER, DAVID EDWARD, McGill University.
SEAMAN, ARLENE R., Instructor in Zoology, Cornell University.
SHAPIRO, ESTHER MAY, Student, Goucher College.
TATUM, ANNE, Student, Rosemont College.
TIEMEIER, OTTO W., Graduate Fellow, University of Illinois.
TUTTLE, RUTH FRANCES, Instructor, Carnegie Institute of Technology.
UBER, VIRGINIA MAE, Assistant in Biology, Pennsylvania College for Women.

PHYSIOLOGY

ABAJIAN, JOHN, JR., Associate Professor of Anaesthesiology, University of Vermont.
BENEDICT, DORA, Student, Harvard Medical School.
BERG, GEORGE G., Graduate Student, Columbia University.
CLARK, CARL CYRUS, Assistant, Columbia University.
CONNELLY, CLARENCE M., Graduate Student, Cancer Fellow, Johnson Foundation, University of Pennsylvania.
CRAPSTER, WIRT PATTERSON, Student, Taneytown, Maryland.
GREGG, JAMES H. Graduate Student, University of Miami.
GREGORY, JOHN C., Graduate Student, Yale University.
GUTHE, KARL FREDERICK, Graduate Student, Harvard University.
HARTING, JANEY, Undergraduate Assistant, Washington University.
HUNTER, FRANCIS ROBERT, Assistant Professor, University of Oklahoma.
JOFTES, DAVID LION, Graduate Student, Tufts College.
JOHNSON, SHIRLEY ALMA, Research Assistant, University of Toronto.
KEMP, NORMAN EVERETT, Instructor in Biology, Wayne University.
LEVIN, ILENE B., Goucher College.
MARSHALL, JEAN MCELROY, Instructor in Physiology, Mount Holyoke College.
MCPHEE, GWELDA S., Assistant in Physiology, Vassar College.
MENDES, ERASMO GARCIA, Assistant Professor, University of Sao Paulo, Brazil.
NARAHARA, HIROMICHI TSUDA, Columbia University.
NELSON, THOMAS CLIFFORD, Graduate Student, Assistant, Columbia University.
ROYS, CHESTER C., University of Chicago.
SASLOW, HERBERT B., Laboratory Instructor and Graduate Student, University of Illinois.
SHULMAN, NAHUM RAPHAEL, Student, Johns Hopkins Medical School.
SILBER, EARLE, Student, University of Maryland Medical School.
ST. GEORGE, ROBERT C. C., JR., Graduate Student, Harvard University.
UMBARGER, H. EDWIN, 239 West Fifth Street, Mansfield, Ohio.

ZOOLOGY

AMBERSON, MARGARET MARY, Oberlin College.
BANNER, ALBERT HENRY, Assistant Professor of Zoology, University of Hawaii.
BERGQUIST, JEANNE BODIN, Barnard College.
BERNSAW, MARGARET IDA, Wilson College.
BINGHAM, BARBARA ALICE, University of Michigan.
CANDELAS, GUSTAVO, Assistant Instructor, University of Puerto Rico.
CATTELL, ELLY, Student, Cornell University.
CHADWICK, JOHN B., Harvard University.
CHIVERS, MIRIAN E., Howard University.
DENTON, WINSLOW CROCKER, Graduate assistant, Cornell University.
EDWARDS, JOHN PARMAN, Student, Drury College.
EHRENTHEIL, SUSANNE JUDITH, Radcliffe College.
EMERSON, JULIA RIPLEY, Student, Wellesley College.
ENDERS, ABBIE GERTRUDE, Student, Swarthmore College.
FELD, EMILY ANN, Student, University of North Carolina.
FERGUSON, EDWARD L., Student, Wesleyan University.
FOLEY, JOSEPH BRENDAN, Graduate student, Yale University.
FOREMAN, DARHL LOIS, Student Assistant, George Washington University.
FULLERTON, ANN ELIZABETH, Student, Western Maryland College.
GEHR, AGNES ROBBINS, Student, Western Reserve University.
GESE, EDWARD CHARLES, Graduate Assistant, New York University.
GESE, PHYLLIS KRING, Student, New York University.
HACKETT, THOMAS PAUL, 573 Purcell Avenue, Cincinnati, Ohio.
HAND, CADET HAMMOND, JR., Student, University of Connecticut.
HOPKINS, AMOS L., JR., Student, Harvard College.
HOPP, WILLIAM BEECHER, Graduate student, Purdue University.
HUMPHREY, JUDITH M., Student, University of Connecticut.
JAKOWSKA, SOPHIE, Fordham University.
KENYON, V. PATRICIA, Graduate assistant, Brown University.
KRAMER, DAVID, Clark University.
LIBERTI, ALFRED V., Graduate student, Fordham University.
LIU CHIEN-KANG, Graduate student, McGill University.
MACHLIS, GERTRUDE RAFFERTY, Assistant in Zoology, University of Illinois.
MEINKOTH, NORMAN AUGUST, Assistant in Zoology, University of Illinois.
MENDES, MARTA VANNUCCI, Assistant Professor, University of Sao Paulo.
MILLER, MARY ELIZABETH, Student, Western Maryland College.
MORRIS, DAVID MARKLAND, JR., Graduate assistant in Zoology, Indiana University.
MOULTON, JAMES MALCOLM, Undergraduate Laboratory assistant, Massachusetts State College.
PETERS, REV. W., Graduate student, Yale University.
POLLENS, NORMAN BURTON, Assistant in Zoology, University of Rochester.
RICE, MARY ESTHER, Drew University.
ROBINSON, EDWIN JAMES, JR., Graduate assistant, New York University.
SANDERSON, MARGARET CRASSONS, Vassar College.
SASLOW, HERBERT B., Graduate assistant, University of Illinois.
SEITNER, PHILIP G., Graduate Assistant, Purdue University.
SMITH, THOMAS C., Student, Oberlin College.
SULLIVAN, REV. THOMAS DONALD, Fordham University.
SWANSON, ANN TERESA, Graduate Assistant, Tufts College.
THOMPSON, MARY JANE, Graduate student, Yale University.
VANHOESEN, DRUSILLA, Student, University of Pennsylvania.
VIVIAN, JANET, Graduate student, Radcliffe College.
WARNER, KATHLEEN LOUISE, Instructor, Mundelein College.
WARTERS, MARY ELLEN, Student, Oberlin College.
WETMORE, KATHERINE BERYL, Student, Radcliffe College.
WILLIAMS, ERNEST E., Columbia University.

4. Tabular View of Attendance

	1942	1943	1944	1945	1946
Investigators—Total	201	160	193	212	267
Independent	132	89	112	138	175
Under instruction	16	19	11	10	29
Library readers	28	35	50	38	38
Research assistants	25	17	20	26	25
Students—Total	74	68	75	96	122
Zoology	36	47	37	55	57
Embryology	24	13	23	23	30
Physiology	6	8	10	13	26
Botany	8	—	5	5	9
Total Attendance	275	228	276	308	389
Less persons registered as both students and investigators	2	6	1		
	273	222	275		
Institutions Represented—Total	126	116	106	124	141
By investigators	83	70	74	100	102
By students	43	41	41	49	56
Schools and Academies Represented					
By investigators	2	2	1	2	2
By students	—	1	2	2	—
Foreign Institutions Represented					
By investigators	—	2	2	1	7
By students	—	—	3	—	5

5. Subscribing and Cooperating Institutions

1946

Cooperating Institutions

Amherst College
Barnard College
Bryn Mawr College
The Catholic University of America
Columbia University
Cornell University
Cornell University Medical College
Duke University
Fish and Wild Life Service, U. S. Department of the Interior
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Johns Hopkins University
Lankenau Hospital Research Institute
Eli Lilly and Company
Massachusetts Institute of Technology
Mount Holyoke College
New York University
New York University College of Medicine
New York University School of Dentistry
New York University, Washington Square College
Oberlin College
Ohio State University
Princeton University
Rockefeller Institute for Medical Research
State University of Iowa
Syracuse University Medical School
Temple University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Rochester
Vassar College
Washington University
Wellesley College
Wesleyan University
Western Reserve Medical School
Wheaton College
Wilson College
Woods Hole Oceanographic Institute
Yale University

Subscribing Institutions

American Philosophical Society (Penrose Fund)
Biological Institute, Philadelphia, Pennsylvania
Brown University
California Institute of Technology
Canisius College
Carnegie Institute of Technology
College of Mount Joseph-on-the-Ohio
Connecticut College for Women
Elmira College
Johns Hopkins Medical School
Lewis College of Science and Technology
Macy Foundation
Memorial Hospital
Miami University
Pomona College
Radcliffe College
St. Anselm's College
St. Michael's College
University of Kansas
University of Texas
University of Toronto
University of Vermont
Vanderbilt University Medical School
Western Maryland College

6. Friday Evening Lectures, 1946

Friday, June 28
Dr. Jane Oppenheimer ...."The Organization of the Teleost Blastoderm."

Friday, July 5
Prof. E. N. Harvey ....."The Effects of High Velocity Missiles on Tissue."

Friday, July 12
Dr. Benjamin W. Zweifach ....."The Relation of Metabolic Derangements of Liver and Kidney to Peripheral Vascular Reactions."

Friday, July 19
Dr. E. W. Dempsey ....."Observations on the Chemical Cytology of Several Mammalian Tissues."

Friday, July 26
Prof. K. S. Cole ....."'A" Bomb and Biology."

Friday, August 2
Dr. E. J. Cohn ....."The Separation of Plasma into Fractions Varying in Chemical and Biological Properties."

Friday, August 9
Dr. Eric G. Ball ....."Biochemical Studies on the Malarial Parasite."

Friday, August 16
Prof. M. H. Jacobs ....."Cell Membranes, Cell Permeability and Cell Volume."

Friday, August 23
Dr. E. J. Boell ....."Biochemical Differentiation during Amphibian Development."

Other Lectures

Thursday, July 18
Kodachrome Motion Picture
C. Lloyd Claff ....."Cardiac Resuscitation—Resuscitation Method after Asphyxia. Heart Defibrillation to Control Ventricular Fibrillation during Heart Surgery."

Thursday, July 25
Dr. Howard Meyerhof ..... "The Crisis in Science Legislation."

Thursday, August 15
Prof. Harlow Shapley ..... "Internationalism in Science."

Wednesday, August 21
Prof. J. H. Bartlett ..... "A Possible Mechanism of Nerve Action."

Saturday, August 31
Dr. Paul S. Galtsoff ..... "The Atom Bomb Test at Bikini."

7. Seminars, 1946

Tuesday, July 9
Dr. Albert Tyler ..... "Inhibition of Fertilization in Sea Urchins by means of Univalent Antibodies vs. Antifertilizin.
Dr. W. A. Robbie ..... "The Cyanide Sensitivity of the Unfertilized Sea Urchin Egg."
Dr. J. E. Kindred ..... "The Effects of Mustard Vesicants on the Hemopoietic Organs."

Tuesday, July 16
Dr. A. F. Bliss ..... "Intermediate Steps in the Visual Cycle."
Dr. A. M. Shanes ..... "The Role of Metabolism in the Injury Potential of Frog Nerve."
Dr. W. H. Price ..... "Adenosine Triphosphatase, Myosine, Actin, and their Relation to the Mechanico-Chemical Coupling of Muscle."

Tuesday, July 23
M. M. Brooks ..... "Oxidation-reduction Studies as a Clue to the Mechanism of Fertilization of Marine Eggs."
C. L. Yntema ..... "An Analysis of Induction of the Auditory Vesicle in the Salamander."
J. T. Bonner ..... "Aspects of Morphogenesis in the Slime Molds."

Tuesday, July 30
Dr. Ernst Scharrer ..... "Chemical Sense and Taste in the Sea Robin, Prionotus."
Dr. Claude A. Villee ..... "Studies of the Respiration of the Imaginal Discs of Drosophila, using the Cartesian Diver Ultramicrorespirometer."
Dr. C. B. Anfinsen and Dr. E. G. Ball ..... "The Action of Naphthaquinone Antimalarials on Respiratory Enzymes."

Tuesday, August 6

Dr. A. R. Whiting
Dr. H. C. George ..... "X-Ray Induced Visible and Dominant Lethal Mutations in Habrobracon Eggs."
Dr. P. B. Armstrong ..... "Specificity of Cholin Esterase."
Dr. T. H. Bullock ..... "The Giant Synapse in the Stellate Ganglion of the Squid as a Physiological-Preparation."

DR. BERTA SCHARRER ..... "The Endocrine Function of the Corpora allata in Insects."

Tuesday, August 13

DR. KATHARINE A. BROWNELL ..... "Evidence of a New Factor from the Adrenal."
DR. F. A. HARTMAN ..... "Hyperactivity of the Adrenal Cortex."
DR. ARNOLD LAZAROW ..... "Studies on the Mechanism of Production of Diabetes with Alloxan."
DR. DOROTHY WRINCH ..... "On the Nature of Biological Specificity."

Tuesday, August 20

DR. C. G. WILBER ..... "A Comparative Study of the Lipids in some Marine Annelids."
DR. C. A. BERGER ..... "Polyploid Mitosis as a Normal Factor in the Development of Allium cepa."
DR. P. W. WHITING ..... "Reproductive Economy in Close-crossed
DR. R. G. SCHMIEDER Species with Haploid Males."
DR. T. U. H. ELLINGER ..... "Hippocrates on Semen and on the Origin of the Child.

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THE PERIOD OF QUIESCENCE IN THE RESPONSE TO LIGHT BY AMOEBA (THE RESPONSE TO LIGHT BY AMOEBA)

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Introduction

As is well known, an amoeba, when subjected to sudden illumination, responds by cessation of movement, and, as this cessation does not take place immediately, an interval exists between the increase in illumination and the reaction, thus giving rise to a reaction-time. As shown in a previous paper (Folger, 1925), the duration of the reaction-time depends on various factors, such as intensity of the light, previous stimulation, physiological condition of the animal. However, the reaction-time does not constitute the whole of the response to light by amoeba. After stopping, the animal remains motionless for some time, then resumes locomotion. The interval during which movement is suspended and which has been designated the quiescent period or period of quiescence is likewise dependent upon numerous factors and must necessarily be included in a complete investigation of the response to light by the organism.

Investigations dealing with a phase in the reaction of light similar to the one just mentioned have not been numerous. Possibly the growth reaction in a mold, as described by Blaauw (1914), may be compared to it. Blaauw was able by means of mirrors to illuminate the spore-carrier of *Phycomyces nitens* on four sides simultaneously, thus bringing about a symmetrical stimulation and avoiding bending. He found that after an illumination of one minute an increase in the rate of growth could be detected about three and a half minutes later, though this reaction-time was subject to much variation, depending both on the individual and on the intensity of the light. The increase in growth was augmented more and more until in some cases it amounted to as much as two hundred or even four hundred per cent of the normal rate. Shortly afterward, however, growth began gradually to slow down until finally it fell below normal, a condition which was of short duration for soon the plant was growing at its usual rate again.

Apparently more closely related to the phase to be considered in *Amoeba* is that encountered in streaming plant cells such as those of *Chara* and *Nitella*, as described by Ewart (1903), who gives an excellent review of previous work on protoplasmic streaming and adds the results of experiments by himself. Unfortunately for work with illumination, the cells of both of these plants contain chloroplasts, and photosynthesis brings in a complicating factor which interferes somewhat with a study of other effects of light. But in the case of several additional stimulating agents, especially mechanical shock, there is a response similar to that given by *Amoeba* to sudden illumination. The response of a streaming cell of *Chara* to mechanical shock, for instance, comprises a cessation followed shortly by a resumption of movement,

and, as Ewart shows, a close relationship exists between the intensity of the stimulating agent and the time during which streaming remains suspended.

Some use has already been made of the period of quiescence in an investigation of the reactions of *Amoeba*. Folger (1926) demonstrated that in response to mechanical shock it becomes longer with increase in the magnitude of stimulation. This paper is concerned with a study of the quiescent period in the response of *Amoeba* to light.

Materials and Methods

Apparatus similar to that used in the experiments to be presented has already been described (Folger, 1925). Light was obtained from a 1000-watt, 112-volt, cylindrical Mazda stereoptican lamp and flashed upon the amoebae by means of the plane mirror of the microscope. Heat rays were eliminated by placing a vessel of distilled water between lamp and mirror. The voltage of the current was about 114, and as the amoebae were placed at a distance of 25 centimeters from the light, they were, consequently, subjected to an illumination of approximately 16,000 meter candles intensity, even after some absorption of light by mirror and water. Active specimens of *Amoeba proteus* were used as experimental animals.

Experimental Results

Mention has been made of the lengthening of the period of quiescence with increase in the magnitude of mechanical shock. In the experiments referred to, the shock was brought about by dropping pieces of wire through a glass tube to the slide on which the amoebae had been placed. Under these conditions the time of application of the stimulus did not vary, it might be described as instantaneous, and the distance through which the weights fell was not changed. Consequently the intensity of the shocks varied with the weights of the pieces of wire, which were all of the same diameter but of various lengths. In the experiments now to be described, the intensity of the stimulating agent, that is, the light, remained unchanged, but it was easy enough to vary the magnitude of the stimulus by altering the time of exposure. The following effects of light on the period of quiescence were investigated: (1) the effect of changing the length of exposure; (2) the effect of altering the time elapsing between exposures; (3) the effect of repeated exposures.

Relation between the period of quiescence and the exposure period

If one keeps the intensity of the light constant but alters the time during which it acts, it is immediately apparent that the quiescent period varies with the time of exposure. This is well illustrated by the results of the experiment recorded in Table I, in which an amoeba was subjected to light for periods ranging from 4 seconds to 45 seconds. A response was obtained in every instance, with an average reaction-time of about 2.6 seconds.[1] As can be seen, it was quite otherwise with the period of quiescence. An exposure of 4 seconds produced a quiescent period of 10.6 seconds; one of 10 seconds, a quiescent period of 18.7 seconds; and one of 15

[1] It is not to be assumed that length of exposure can have no effect on the reaction-time, but to produce a reaction-time essentially different from that given above would require a much shorter exposure than any used in this experiment.

seconds, a quiescent period of 24 seconds. Thus the period of quiescence increased with increase of exposure and this continued until the exposure amounted to 45 seconds, when the period of quiescence was 42 seconds.

Further analysis brings out other relationships between exposure and quiescent periods. In the final column is shown the difference obtained by subtracting the former from the latter. With an exposure of 4 seconds the difference amounted to 6.6 seconds. With an exposure of 10 seconds it was 8.7 seconds, and with an exposure of 15 seconds it was 9 seconds. Thus far the rate of increase has been greater in the period of quiescence than in the exposure period, but this did not continue indefinitely, for with an exposure of 20 seconds the difference had fallen to

TABLE I

Showing increase of the period of quiescence with increase of exposure period. Time indicated by seconds.

Exposure period (s)	Number of tests	Period of quiescence (t)			t-s
		Maximum	Minimum	Average	
4	5	14	7	10.6	6.6
10	3	20	17	18.8	8.7
15	3	30	21	24.0	9.0
20	3	32	24	27.0	7.0
25	2	29	27	28.0	3.0
30	2	32	32	32.0	2.0
45	1			42.0	−3.0

7 seconds, with an exposure of 30 seconds it was 3 seconds, and with an exposure of 35 seconds it was 2 seconds. The exposure period is now increasing faster than the period of quiescence, and the ultimate result is shown at an exposure of 45 seconds, when the difference amounted to − 3 seconds. In other words, the exposure period has become longer than the period of quiescence. It has already been stated that the amoeba ceased to move after it was in illumination for about 2.6 seconds. So, when exposed for 45 seconds, it stopped in 2.6 seconds and resumed movement in another 42 seconds, 0.4 of a second before the light was turned off. Obviously an exposure greater than 45 seconds would not have called forth a longer period of quiescence, since the amoeba would still have stopped and then resumed movement within about 44.6 seconds after the light was turned on.

Time required for recovery from the effects of sudden illumination

Reaction by *Amoeba* to light or to mechanical shock is, as previously stated, followed by a refractory period, which may be either absolute or relative; that is, the amoeba may refuse to react to a second stimulus, or it may react but with an altered response. Partial recovery from the effects of either kind of stimulus is indicated by a reaction-time that is longer than that obtained after complete recovery (Folger, 1925, 1926). The effect of partial recovery from sudden illumination on the period of quiescence is illustrated by the results of the experiment shown in Table II.

TABLE II

Showing the influence upon the period of quiescence on the lack of recovery from the effects of previous stimulation. Light turned on and left on until amoeba resumed locomotion.

Time in dark previous to exposure	Number of tests	Period of quiescence (sec.)		
		Maximum	Minimum	Average
4 seconds	4	No reaction		
10 seconds	4	30	18	22.5
30 seconds	6	40	15	24.8
1 minute	6	60	25	36.7
2 minutes	6	75	40	54.2
10 minutes	1			175.0
30 minutes	1			220.0
2 hours	1			218.0

In this experiment a single amoeba was used and was subjected to a series of tests in each of which it was first exposed to light for 1 minute, then to darkness for 3 minutes, and again to light, this time for 30 seconds. This procedure was intended to bring about a condition of uniformity. Following the 30-second exposures the light was turned off for intervals varying in the several trials from 4 seconds to 2 hours. Finally in each case the amoeba was then exposed to illumination and left exposed until the characteristic response was completed by the resumption of flow. As shown in the table, no reactions were obtained when 4 seconds were allowed for recovery. Ten seconds permitted a recovery sufficient to obtain reactions in every instance, with an average period of quiescence in 4 trials of 22.5 seconds. Thirty seconds for recovery resulted in an average period of quiescence of 24.5 seconds; 1 minute for recovery, in an average period of quiescence of 36.7 seconds; and 2 minutes for recovery, in an average period of quiescence of 54.2 seconds. Ten minutes, 30 minutes, and 2 hours for recovery were followed by periods of quiescence of 175 seconds, 220 seconds, and 218 seconds, respectively. Clearly the period of quiescence became longer as the amoeba recovered from the effects of previous stimulation, reaching a maximum when recovery was complete. Evidently under the conditions of the experiment complete recovery was not attained until after 10 minutes but before 30 minutes had elapsed. This contrasts with the 1 or 2 minutes necessary to obtain a minimum reaction-time (Folger, 1925, Alsup, 1937).

A few further words of explanation are necessary for a proper evaluation of the results just given. When an amoeba is exposed to light and ceases to move, the stoppage is generally sudden, permitting accurate timing with a stopwatch. If the exposure period is not too long or if too much time has not elapsed between stimuli, the resumption of movement also occurs with sufficient abruptness to be timed with a fair degree of precision. However, when a strong light is combined with sufficient time for complete or almost complete recovery from the effects of previous stimulation and with a long exposure period, expecially with one so long that the amoeba begins to move while the light is still acting, the resumption of flow cannot be ascertained with the same accuracy. Under these conditions the first indication of movement is usually an extrusion of a number of small pseudopodia, followed by an erratic flow, first in one direction, then in another. Gradually these

pseudopodia lessen in number, movement becomes stronger, until finally the amoeba is again flowing briskly. If, now, one considers the first indication of movement as the end of the period of quiescence, it is easily determined. But if he assumes that this period is not completed until the amoeba has resumed the more vigorous movement that obtained before stimulation, he must exert a judgement, and the period can not be timed with the same degree of accuracy. In the present instance the period of quiescence was interpreted as ending when the protoplasmic flow became as strong as it was before stimulation, but even if the first indication of movement had been chosen as the final point in the reaction, while the higher figures in the preceding table would have been altered by several seconds, in the very long exposures even by as much as 25 or 30 seconds, the general aspect of a curve drawn from them would not be materially changed.

The effect of repeated stimulation

Repeated stimulation by light, with a short time intervening between exposures, brings out another peculiarity in the behavior of amoeba, which is exemplified by the results of the experiment summarized in Table III. This table records averages

TABLE III

Showing decrease in period of quiescence with repeated exposures. Each amoeba subjected to darkness for at least 10 minutes and then given 10 successive 3-second exposures to light, with intervals of 1 minute in darkness between exposures. Number of animals in cultures 1, 2, 3, were 11, 13, and 16, respectively.

Reaction number	Average period of quiescence (sec.)			General average
	Culture No. 1	Culture No. 2	Culture No. 3	
1	18.5	21.6	14.3	18.1
2	17.8	18.6	14.4	16.9
3	12.6	14.0	14.6	13.7
4	11.6	14.3	12.6	12.8
5	10.4	12.4	12.4	11.7
6	8.8	11.9	10.1	10.3
7	10.8	11.5	11.9	11.4
8	10.4	9.4	10.8	10.2
9	8.8	8.2	9.9	9.0
10	10.3	10.1	8.8	9.7

of the reactions of from 11 to 16 amoebae from each of 3 cultures. Each amoeba was subjected to darkness for at least 10 minutes and then given 10 successive 3-second exposures to light, with intervals of 1 minute in darkness between exposures. Though there were variations among the animals from the same culture and even more among animals from diverse cultures, the general behavior was the same in every case and is reflected in the total averages given in the table. The average period of quiescence for the first exposure amounted to 18.1 seconds, for the second exposure to 16.9 seconds, for the third exposure to 13.7 seconds. As one may see, this decline continued until the sixth exposure, when the period of quiescence was 10.3 seconds. In the 4 succeeding trials at no time did it fall far below 10 seconds

and on the tenth exposure amounted to 9.7 seconds. Even when further tests were added, as they were in some instances though the results are not given in the table, there was no further reduction in the period of quiescence.

This diminution of the period of quiescence with repeated exposure is somewhat reminiscent of the phenomenon known as treppe, to be met with in the physiology of muscle.

Discussion

The response to light that has been under consideration is intimately related to locomotion and must perhaps in final analysis be explained in terms of amoeboid movement. According to Mast (1926), an amoeba consists essentially of an inner fluid plasmasol and an outer solid plasmagel, which is surrounded by a very thin elastic surface membrane, the plasmalemma. The plasmasol is hypertonic and the plasmagel and plasmalemma act as semipermeable membranes, resulting in the development of an osmotic pressure in the plasmasol and a stretching of the plasmagel, especially at these points where it is weakest, where the pseudopodia are formed. Locomotion, according to Mast, involves a continuous change from plasmasol to plasmagel at the anterior end of the animal and from plasmagel to plasmasol at the posterior end. For further analysis of amoeboid movement one is referred to Mast's paper. For our purpose it will suffice to point out that when it is moving, the amoeba must be in a state of dynamic equilibrium. Illumination might cause an increase in the elastic strength of the plasmagel, especially at the tip of the advancing pseudopodium (Mast, 1932), bringing about a temporary breakdown in the established equilibrium, with consequent cessation of forward movement. Shortly an equilibrium is reformed and the animal again moves. Since experiments described in the preceding section have shown that stimulation applied shortly after the resumption of locomotion either fails to bring about a reaction or is followed by a reaction with a shortened period of quiescence, it is apparent that the new equilibrium is by no means at the same level as that which was in existence before the cessation occurred. Otherwise one would expect identical responses when the one stimulation is followed by another of equal strength. The experiments show, moreover, that the original level is gradually restored since a stimulus of a given intensity will finally elicit a second response of exactly the same magnitude as that which it called forth at first. Obviously, the time during which the disturbed equilibrium is reverting to the original level constitutes the refractory period.

Summary

1. *Amoeba proteus* reacts to sudden illumination by cessation of movement. The time during which it is motionless has been designated the period of quiescence.

2. The period of quiescence becomes longer with extension of the time of exposure to illumination. At first the period of quiescence increases more rapidly than the exposure period, but soon the rate of increase of the latter becomes the greater, with the final result that the amoeba begins to move while the light is still on.

3. Stimulation by light is followed by a refractory period, which may be absolute

or relative; that is, the amoeba may either refuse to respond to a second stimulus, or it may react but with an altered response. Incomplete recovery from the effects of previous stimulation results in a period of quiescence that is shorter than that obtained after complete recovery.

4. If an amoeba is repeatedly exposed to illumination at intervals of a minute, the period of quiescence is at first relatively long, but becomes progressively shorter, arriving at a minimum after about 6 minutes.

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A METHOD FOR REMOVAL OF THE SINUS GLAND FROM THE EYESTALKS OF CRUSTACEANS[1]

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An increasingly large number of endocrine effects has been attributed to the crustacean sinus gland within the last decade (see reviews by Kleinholz, 1942 and by Brown, 1944). Efforts to determine the role played by these reported endocrine effects in the normal physiology of the animal have proceeded along conventional paths. Although investigators have applied the classical methods of endocrinology in determining in crustaceans the endocrine function of a suspected organ, i.e., deficiency and replacement experiments, the deficiency experiments have not been completely adequate; at times the uneasy feeling arises that some of the reported experimental results may be artifacts.

This situation has come about because removal of the sinus gland, located between the 2nd and 3rd optic ganglia of the eyestalk, is most simply accomplished by ablation of both eyestalks. As has already been pointed out (Kleinholz 1938, 1942), such surgery removes at the same time a not inconsiderable portion of the central nervous system, the four large ganglia located in each stalk. Control experiments to eyestalk ablation, in which antennae or other appendages are removed to simulate equivalent nerve injury or traumatic damage to the experimental animal, are thus not qualitatively comparable. The extent to which these ganglia in the eyestalk are centers of reflex activity, other than visual, has apparently not been adequately determined; the possibility exists that these ganglia of the eyestalk may function as inhibitory centers of nervous activity as does the brain itself (Bethe, 1898; Roeder, 1937).

In recognition of the fact that the most facile surgical procedure is not necessarily the most critical or advisable one, is the growing realization that deficiency experiments in the physiological study of the sinus gland must be made after removal of the sinus gland alone, with as little attendant injury to the nerve ganglia as is possible. Technically, this is not always feasible, especially among the smaller crustaceans. With the larger macrurans and brachyurans, however, surgical excision of the sinus gland is possible by the application of a little ingenuity. Brown (1942) has described such a method for removing the sinus gland from the eyestalk of the crayfish. The procedure described in this note allows removal of the sinus gland with perhaps less disturbance or injury to the eyestalk than the method of Brown. As described here, the method represents the adoption, over several years, of slight modifications in technique to solve the various difficulties that ap-

[1] This work was started in 1941 with the aid of a grant from the Permanent Science Fund of the American Academy of Arts and Sciences. Continuation after the war was made possible by a John Simon Guggenheim Memorial Fellowship. I am indebted to the Chairman of the Division and to the Director of the Biological Laboratories of Harvard University for the many courtesies and facilities extended me.

peared during removal of the sinus gland; other investigators who may attempt such surgery will probably discover alternative improvements in the method.

Such gland removal has been performed on the crayfish and the lobster, the latter animal being easier to operate upon because of its larger size. The steps outlined below apply more readily to the crayfish.

It was early found necessary to have the animal anesthetized, because with an animal that struggled during the more delicately manipulative steps of gland removal, serious damage of the nervous components of the eyestalk would result. Trial of several methods of anesthesia (chloretone, cold) were unsatisfactory; diluted chloroform water proved to be excellent for the purpose. This was prepared by saturating 100 ml. of water with 1 ml. of chloroform by vigorous shaking, allowing the mixture to stand for 2 or 3 minutes so that undissolved chloroform droplets would settle, and pouring the supernatant into a large beaker to which was added about 500 ml. of water. The crayfish was immersed in this dilute chloroform solution for 1–3 minutes; much longer immersion gave too deep anesthesia which was not always followed by successful recovery. After removal from the chloroform solution the animal was washed briefly in running tap water and fastened to an operating board.

The operating board was a rectangular piece of plywood, about 3½ inches by 6 inches in its dimensions, with a short stout elastic rubber band encircling each end. The anesthetized animal is held in place by inserting the chelipeds under the elastic band at one end, and the abdomen under the rubber band at the other end. After thus fastening the animal, a ligature of cotton thread is loosely tied around the non-skeletogenous base of the eyestalk. This serves as an effective hemostat during later steps of gland removal; excessive bleeding can be prevented or reduced by tightening the ligature. A small piece of modelling clay is used as a wedge between the stalk and the rostral orbit (Fig. 1); this keeps the eyestalk in a fixed position and prevents damage to the retina that might ordinarily result from pressure of the stalk against the carapace or rostral base during subsequent trephining.

Trephination of the exoskeleton is accomplished with the aid of drills of appropriate size. For the eyestalk of *Astacus trowbridgei,* used in this study, a trephine measuring about 2 mm. in outside diameter was satisfactory.[2] The centerpin of the trephine was placed, as shown in Figure 2, slightly posteriorad of the long axis of the stalk and approximately in the center of the non-retinal portion. Rotation of the drill would cut a circumscribed disc of the exoskeleton (Fig. 3). It was found helpful to perform the trephination with the aid of a binocular dissecting microscope, using a magnification of about 18–20 ×, and employing a source of bright illumination such as an adjustable-focus microscope lamp. Once the disc of exoskeletal material had been cut thru, it was removed after carefully separating it from the underlying hypodermis by means of a fine knife. A series of knives of various sizes and shapes was prepared by sharpening steel needles on a hone and mounting them in convenient handles. The excise skeletal disc was placed in normal saline, to be used later in closing the opening. The exposed hypodermis, readily recognized by its numerous red chromatophores, was dissected free and also stored in saline. When this has been done and the bright light focused on the operative field, the nervous portion of the eyestalk and the outline of the sinus gland can

[2] Drills of assorted sizes have been made for me by Mr. H. G. Bliss, 507 Park Avenue, Herkimer, New York.

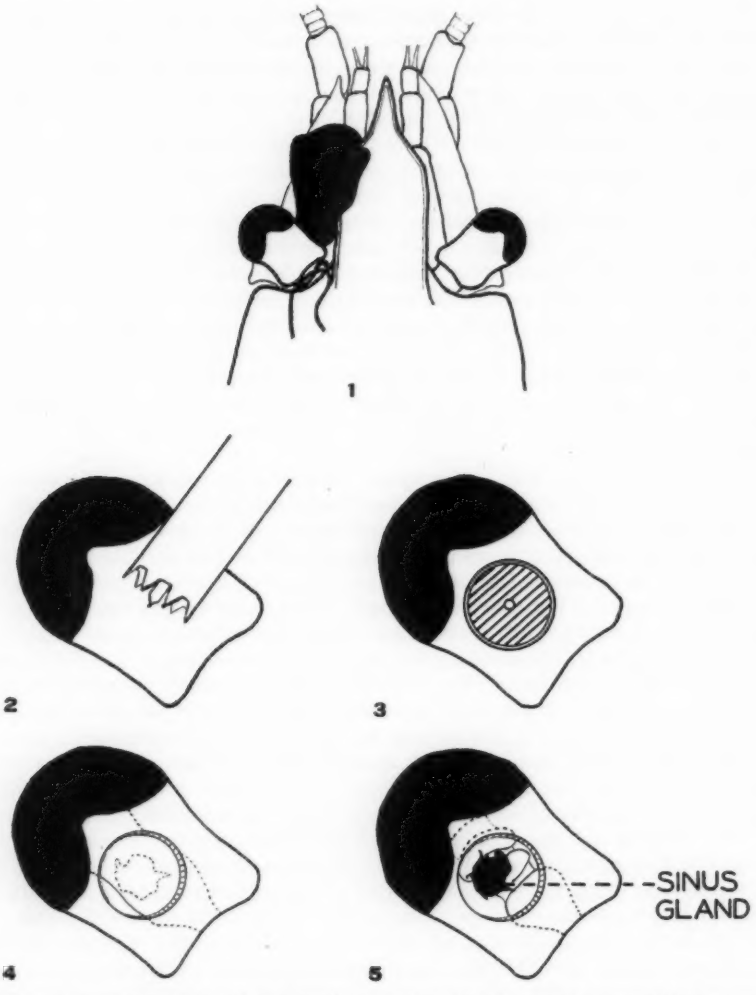

FIGURE 1. The cephalic end of a crayfish, showing the left eyestalk prepared for removal of the sinus gland. A ligature is first loosely tied around the base of the eyestalk; this is tightened only before trephination is started. A piece of modelling clay holds the stalk in position and avoids the risk of crushing the retinal portion against the rostrum or carapace.

FIGURE 2. The left eyestalk viewed from the dorsal side, showing the location of the trephine to expose the area of the sinus gland.

FIGURE 3. The trephined area of the exoskeleton shown in diagrammatic form.

FIGURE 4. The trephined disc of skeletal material has been removed; the hypodermis, lying directly underneath the exoskeleton has also been excised. In dotted outline are shown the optic ganglia and the sinus gland, enclosed in a thin transparent sheath. The muscles of the eyestalk are not shown.

FIGURE 5. The connective tissue sheath has been removed, showing the gland and its processes located between the 2nd and 3rd optic ganglia.

be detected with the binocular microscope. Some of the muscles of the stalk lie alongside the nerve ganglia, but can be readily separated from the latter by a fine knife. The operative field at this point is diagrammed in Figure 4. A thin, transparent sheath encloses the optic ganglia and the sinus gland. This sheath is carefully slit, and the incision extended around the margin of the trephined area. In the light of the microscope lamp the sinus gland now appears as a bluish-white structure, quite easily distinguishable from the nervous portion of the stalk (Fig. 5).

The sinus gland is located between the second and third optic ganglia, in a somewhat dorso-posterior position with regard to the optic tract. In the crayfish (*Cambarus* and *Astacus*) it is not as compact a structure as is found in many of the marine crustaceans; a few filaments or processes of the gland are found to extend to adjacent ganglia; in the eyestalk of *Astacus* this seems to be less pronounced than in *Cambarus*.

After the gland has been exposed, the processes and body of the gland are carefully separated from the optic ganglia by a knife, and the entire structure removed with the aid of watchmaker's forceps and iridectomy scissors. The operative field is examined with the binocular microscope to verify complete excision; fragments of the filaments that have remained behind can be removed with the forceps.

Following removal of the sinus gland, the hypodermis, previously excised and saved in saline, is replaced to fill the gap and the trephined disc of skeletal material is fitted into the opening in the exoskeleton of the eyestalk. The groove between the margin of this opening and the rim of the disc is filled with fibrin foam. This material originally consisted of samples from the laboratory of Professor E. J. Cohn of the Harvard Medical School; later, commercial preparations, "Gelfoam, No. 12" manufactured by the Upjohn Company, proved equally satisfactory. The ligature around the base of the eyestalk is removed. The slight oozing of blood that follows in the trephined area soon leads to clotting in contact with the fibrin foam. To facilitate this clotting and the desired fixing-in-place of the skeletal disc, the animal is removed from the operating board and placed in a vessel containing water to a depth not much greater than an inch; after 2–3 hours the operated animal is replaced in its tank of deeper water, with little risk that the trephined disc will be loosened and the area of operation exposed.

In practice I have found that the operation from anesthesia to gland removal and replacement of the disc requires about 15 minutes. The initial attempts required as long as an hour, until some facility in the procedure was attained.

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THE COMPARATIVE TOLERANCES OF SOME FOULING ORGANISMS TO COPPER AND MERCURY *

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The ability of marine organisms to attach to surfaces coated with ship-bottom paints depends on the toxicity of the surface and the sensitivity of the organisms to the toxic. Pyefinch (1946) presents a table showing the sensitivity of various organisms to toxics determined primarily from analysis of their settlement on toxic surfaces at Millport, Scotland. In the present studies carried out in the Biscayne Bay area, Florida, the tolerances of several common fouling organisms to copper and mercury have been compared by observations of the sequence of attachment to antifouling paints of moderate toxicity, to series of paints of graded toxicity, and to nontoxic surfaces adjacent to antifouling paint.

Results

An indication of the relative tolerance of various organisms to toxic paints can be obtained by observing the frequency of their attachments to paints which are beginning to fail after long exposure. It may be expected that the most tolerant forms will be the first to attach. The organisms attaching earliest to each of 662 antifouling paints exposed at Miami Beach and Tahiti Beach, Florida, were recorded with the following result:

Barnacles	67%
Encrusting bryozoans	18
Hydroids	9
Erect bryozoans	5
Tunicates	1
Tubeworms	< 0.1
Sponges	< 0.1

The order of attachment depends not only upon the tolerance of the organisms to the toxics, but also upon their seasonal occurrences. For example, hydroids and those forms following them in the list attach first only when barnacles and encrusting bryozoans are absent from the fouling population. Encrusting bryozoans rank below barnacles because they are absent during a large part of the year, while barnacles are present at almost all times. One of the encrusting bryozoans, *Watersipora cucullata*, appears to be somewhat more tolerant to copper than the most tol-

* Contribution No. 385 from the Woods Hole Oceanographic Institution. The observations described were made during an investigation of fouling by the Woods Hole Oceanographic Institution under contract with the Bureau of Ships, Navy Department, which has given permission for their publication. The opinions presented here are those of the author and do not necessarily reflect the official opinion of the Navy Department or naval service at large. The author is indebted to Dr. B. H. Ketchum and Dr. A. C. Redfield for helpful advice and criticism.

erant barnacle species, *Balanus amphitrite*, since it frequently attached earlier when both organisms were present.

The larva of a fouling organism which is unable to attach to a toxic paint surface may attach to a more resistant form which has already become established on the surface. Following this the adult may then spread over the paint surface. The relative tolerances of some fouling organisms to the toxic surfaces can frequently be assessed from these conditions of attachment.

The barnacles, *Balanus amphitrite* and *Balanus improvisus*, and the encrusting bryozoan, *Watersipora cucullata*, were found attached directly to paint surfaces. Attaching to and growing on these forms were *Schizoporella unicornis*, *Anomia* sp., *Bugula avicularia*, *Pennaria tiarella*, *Lepas anatifera* and tunicates. Examples of several of these organisms which were unable to attach to antifouling paints, but which attached to other forms on the paint, are shown in Figures 1–5.

A more precise evaluation of tolerance is given by comparing the frequency of attachment of different organisms on a surface of moderate toxicity with their frequency on a nontoxic control surface. A paint of moderate toxicity will allow attachment only of the forms having the highest tolerance to the paint toxics. Glass panels coated on one side with a paint in which cuprous oxide served as the toxic were exposed for periods of one month. The uncoated side served as a control. The use of the glass panel gave both surfaces the same color, thus eliminating any differential effect of color on attachment. Table I shows the numbers of barnacles

TABLE I

Total number of barnacles attached to 33 nontoxic and toxic surfaces (80 square inches in area) exposed for one month at intervals between October 1944 and May 1946

Barnacle species	Barnacles on nontoxic surface		Barnacles on toxic surface	
	Total	% of total	Total	% of total
Balanus improvisus	17,959	89.7	15	9.4
Balanus amphitrite	1,503	7.5	145	90.6
Balanus eburneus	564	2.8	0	0.0

of three species collected on these two surfaces. The total numbers of barnacles attaching to the toxic paint surface are less than one per cent of those growing on the nontoxic control. *Balanus amphitrite* comprises over 90 per cent of the population on the toxic surface, being the only barnacle species on most panels, though it is only 7.5 per cent of the population attaching to the control surface. In contrast *Balanus improvisus* makes up 90 per cent of the total attachment to the nontoxic surface but comprises only 9.4 per cent of the population on the paint. *Balanus eburneus* is completely inhibited from the toxic surface. It is thus evident that each of these three species of the same genus demonstrates a different degree of tolerance to the copper paint.

In addition to the barnacle fouling the only other significant attachment to this paint surface was the encrusting bryozoan *Watersipora cucullata* and the green alga *Enteromorpha* sp. Both of these species attached only during the periods of their maximum abundance. On the nontoxic panels a total of twenty species of fouling

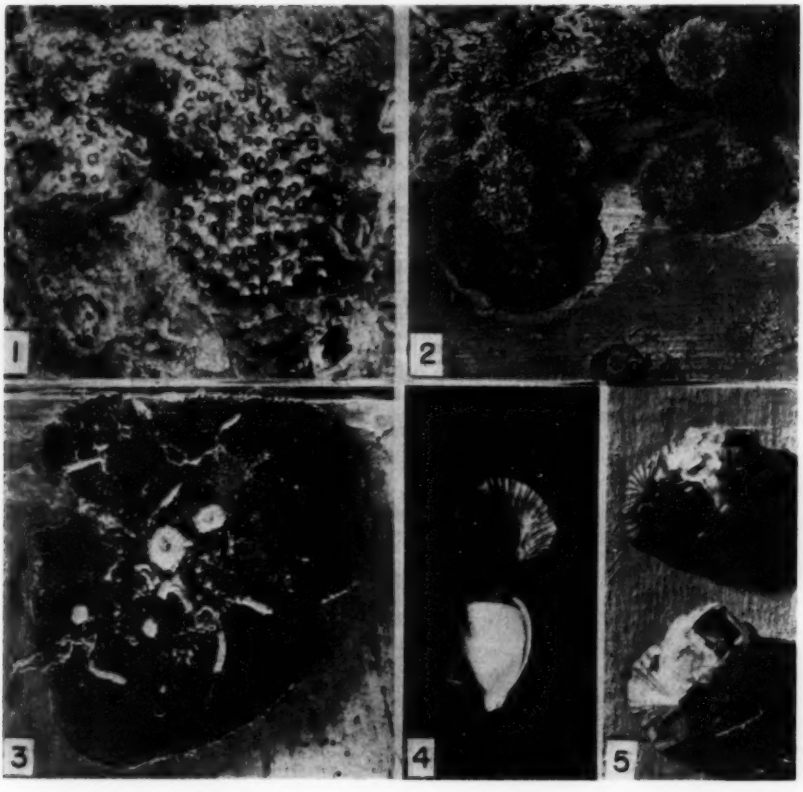

PLATE I

FIGURE 1. A speckled tunicate limited in attachment to barnacles and *Anomia* sp. without spreading onto the paint surface.

FIGURE 2. *Bugula avicularia* attached to the encrusting bryozoan, *Watersipora cucullata*, which is growing directly on the paint.

FIGURE 3. Barnacles attached to *Watersipora cucullata* which developed directly on the paint surface. Tubes of *Dasychone conspersa* are also attached on *W. cucullata*.

FIGURE 4. Gooseneck barnacle, *Lepas anatifera*, attached to *Balanus amphitrite* which attached to an otherwise effective antifouling paint on a ship's bottom.

FIGURE 5. Two examples of *Balanus improvisus* which attached to the more resistant barnacle, *B. amphitrite*. Photographed after removal from the paint surface.

organisms attached during the period covered by these observations. These must be considered to be less tolerant than the four organisms which attached to the paint surface.

In order to establish the relative tolerances to copper of the more sensitive fouling forms a set of paints containing graded amounts of copper flake pigment was prepared. The copper leaching rates of these paints were measured as described by

Ketchum, Ferry, Redfield, and Burns (1945) and were found to vary in proportion to the pigment content and to decrease with the time of exposure. This series thus provided graded toxicities.

The relative tolerance of attaching organisms could be judged by the time of exposure elapsing before they attached to any one member of the series or by the number of members of the series which were fouled at any one time. The paints were applied to 8 × 10-inch weldwood panels which were immersed at Miami Beach and Tahiti Beach, Florida. The composition of the paints and the time of exposure before various fouling forms attached are given in Table II.

TABLE II

Number of months of exposure prior to appearance of fouling on painted panels immersed in the sea at Miami Beach, Florida (May 2, 1945–March 2, 1946), and at Tahiti Beach, Florida (May 2, 1945–September 2, 1945). The paints contained graded amounts of copper flake in a vehicle consisting of equal parts, by weight, of polyvinyl butyral and rosin.

Paint number Copper content, per cent dry weight	AF12 90	121 67	122 45	123 30	124 23	125 14
Miami Beach						
Balanus amphitrite	4	3	3	2	1	1
Watersipora cucullata	9	9	8	2	2	1
Balanus improvisus	a	a	a	a	2	1
Tahiti Beach						
Polysiphonia sp.	3	2	2	2	2	2
Balanus amphitrite	b	b	4	4	3	1
Hydroides parvus	b	b	b	4	4	3

a. None of this species had attached to these paints after 10 months of exposure.
b. None of these species had attached to these paints after 4 months of exposure.

At Miami Beach only three fouling organisms attached to the copper paints, two species of barnacles and the encrusting bryozoan *Watersipora cucullata*. Throughout the period of exposure there was no marked seasonal variation in the incidence of barnacles. However, seasonal fluctuation in the incidence of *Watersipora cucullata* accounts principally for the nonattachment of this organism between the second and ninth months of immersion of the paints. Its absence from the paints is, therefore, not indicative of its sensitivity to the paint toxic.

The exposure at Tahiti Beach, Florida, indicated that, on these copper paints, a red alga of the genus *Polysiphonia* is more tolerant, and that the tube worm, *Hydroides parvus*, is less tolerant than *Balanus amphitrite*.

Some species of fouling were too sensitive to attach even to the paints of lowest toxicity. To ascertain their relative tolerance of paint toxics their sequence of attachment was noted on nontoxic areas adjacent to paint surfaces. Observations by Miller (1946) on the diffusion of toxics from antifouling paints indicate that toxic gradients exist, decreasing from the paint edge. To provide nontoxic areas for attachment adjacent to the paint surfaces, strips 5, 10, and 20 mm. in width were left free of paint on the panels of the graded toxicity series.

At both exposure locations colonial tunicates attached to the widest bare strips on the paints of lowest toxicity, AF124 and AF125, by the second month of immersion.

By the third and fourth months of exposure hydroids had attached to the initial fouling on these nontoxic areas. These observations indicate that tunicates are more tolerant than hydroids to copper but less tolerant than those forms able to attach directly to the paint surface.

From the preceding observations several fouling forms can be arranged in order of decreasing tolerance to copper as follows: *Polysiphonia* sp., *Watersipora cucullata*, *Balanus amphitrite*, *Balanus improvisus*, *Balanus eburneus*, *Hydroides parvus*, tunicates and hydroids.

In the examination of antifouling paints employing mercury pigments or mixtures of mercury and copper pigments as the toxic ingredients, it was noted that certain of the fouling organisms appeared to be more sensitive to mercury than to copper while for others the reverse was true.

To establish the comparative tolerance of different species to mercury a series of paints containing mercurous chloride in graded concentration was made. The vehicle was the same as that used for the copper series. The mercury content of these paints was equal in weight to the corresponding member of the copper series. These paints were immersed simultaneously and at the same locations as the copper paints. The extent of exposure before fouling attached to the paint surfaces of the AF12 mercury series is shown in Table III.

TABLE III

Number of months of exposure prior to appearance of fouling on painted panels immersed in the sea at Miami Beach, Florida (May 2, 1945–March 2, 1946), and at Tahiti Beach, Florida (May 2, 1945–September 2, 1945). The paints contained graded amounts of mercury, added as mercurous chloride, in the vehicle described in Table II.

Paint number Mercury content, per cent dry weight	AF12 90	121 67	122 45	123 30	124 23	125 14
Miami Beach						
Balanus amphitrite	9	9	2	2	2	1
Balanus improvisus	a	a	a	4	9	2
Bugula neritina	a	a	a	2	6	1
Watersipora cucullata	a	a	a	6*	a	4*
Tahiti Beach						
Polysiphonia sp.	b	b	b	3	3	1
Balanus amphitrite	b	b	b	b	2	2

a. None of these species had attached to these paints after 10 months of exposure.
b. None of these species had attached to these paints after 4 months of exposure.
* Single specimen not present on following month.

At Miami Beach four organisms attached to this series of paints. The most resistant of these was *Balanus amphitrite*, followed in order by *B. improvisus* and *Bugula neritina*. *Watersipora cucullata*, which was found to be quite tolerant of copper, attached to only two of these paints, and in neither case was the attachment secure. In contrast, *Bulgula neritina* was able to attach directly to the mercury paints but was never found on any of the comparable copper paint surfaces. At Tahiti Beach *Polysiphonia* sp. was found to be more tolerant than *Balanus amphitrite* to the mercury paints.

Photographs of two of the copper and two of the mercury paints of these series, after nine months of exposure at Miami Beach, are shown in Figure 6. The ability of *Watersipora cucullata* to attach to the copper paints and its absence from the comparable mercury paints are shown. Both of the copper paints and one of the mercury paints are fouled with *Balanus amphitrite*.

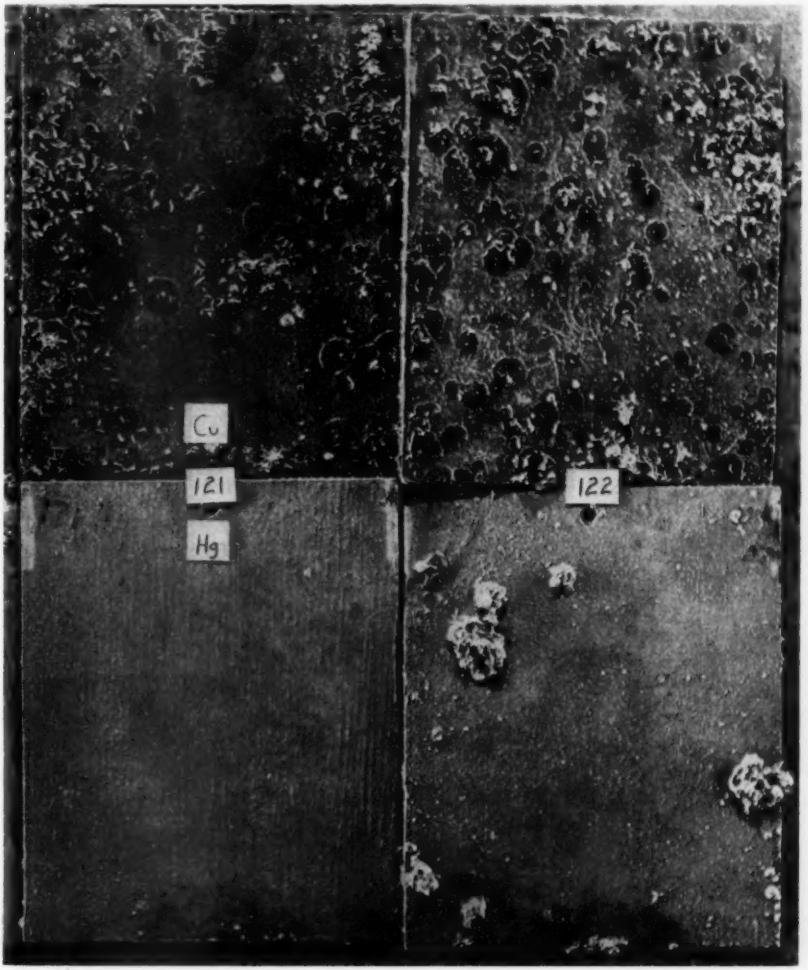

PLATE II

FIGURE 6. Comparison of the attachment of *Watersipora cucullata* to paints pigmented with copper and mercury after nine months of exposure. *W. cucullata* appears on the copper series (top panels) as the dark disc-like organisms.

Nontoxic strips on panels otherwise coated with the mercury paints permitted attachment of the more sensitive fouling forms. The more resistant forms mentioned above always attached first to these nontoxic areas. In addition, *Watersipora cucullata*, *Anomia* sp., hydroids and tunicates invaded the unpainted areas at Miami Beach. At Tahiti Beach *Enteromorpha* sp. and *Hydroides parvus* attached in the order given.

These exposures indicate the following order of decreasing tolerance of some fouling organisms to mercury as a paint toxic: *Polysiphonia* sp., *Balanus amphitrite*, *Bugula neritina*, *Balanus improvisus*, *Watersipora cucullata*, *Anomia* sp., *Enteromorpha* sp., *Hydroides parvus*, hydroids and tunicates.

From the results of the various methods described above the comparative tolerance of several fouling organisms to copper and mercury has been evaluated. The results are given in Table IV. The organisms listed as attaching to adjacent

TABLE IV

The comparative tolerance of some fouling organisms of the Biscayne Bay area, Florida, to copper and mercury employed as pigments in antifouling paints. The lists are arranged in approximate order of decreasing tolerance.

	Copper	Mercury
Organisms found attached to toxic paint surfaces	*Polysiphonia* sp. *Watersipora cucullata* *Balanus amphitrite* *Enteromorpha* sp. *Balanus improvisus* *Balanus eburneus* *Hydroides parvus*	*Polysiphonia* sp. *Balanus amphitrite* *Bugula neritina* *Balanus improvisus* *Watersipora cucullata*
Organisms found mainly attached to nontoxic areas adjacent to paint surfaces	*Anomia* sp. *Bugula neritina* *Bugula avicularia* *Lepas anatifera* tunicates hydroids	*Anomia* sp. *Enteromorpha* sp. *Hydroides parvus* hydroids tunicates

nontoxic surfaces include those which may on occasion be found on paints of low toxicity but which generally do not attach directly to the paint surface. The exact position in the order of decreasing tolerance, for all but the first three organisms in each column, requires further verification.

DISCUSSION

Relatively few of the sessile species in the Miami area which attach to neutral surfaces are able to attach to antifouling paints. Even paints of such low toxicity that they become completely covered permit the attachment of only four or five forms. Many less resistant fouling organisms are excluded from the paint surface. Effective antifouling paints must be designed to prevent the attachment of the few most tolerant forms.

The species most tolerant of copper are different from those most resistant to mercury. The conflicting opinions which have existed concerning the relative

efficiencies of copper and mercury compounds as paint toxics may depend largely on the species of fouling organisms present at the particular testing location.

The present results and those given by Pyefinch (1946) agree in showing that *Enteromorpha* and *Polysiphonia* are among the more resistant forms, and *Anomia* and tunicates have low tolerance to paint toxics. Tubularian hydroids were noted only as secondary fouling at Miami, though Pyefinch rates them more resistant than *Balanus* and equal to *Enteromorpha*. A direct comparison of the results with *Balanus* is not feasible since the three species found in Miami showed marked differences in toxic sensitivity. None of these species are found at Millport where Pyefinch's studies were made. Pyefinch lists a greater variety of algae, including diatoms, which were not included in the investigations at Miami.

Summary

The comparative toxic tolerance of several fouling organisms was based on the sequence of their attachment to copper and mercury antifouling paints. A red alga, *Polysiphonia* sp., and the barnacle, *Balanus amphitrite*, were the most tolerant and attached to copper and mercury paints before other organisms. The encrusting bryozoan, *Watersipora cucullata*, was found to be slightly more tolerant to copper but considerably less tolerant to mercury than the above two forms. Less tolerant forms were *Balanus improvisus*, *Hydroides parvus*, *Bugula neritina*, *Anomia* sp., *Enteromorpha* sp., tunicates and hydroids. Other species, though attaching to nontoxic surfaces, were never found on the toxic paints.

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SYSTEMATIC SEROLOGY AMONG CERTAIN INSECT SPECIES

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Introduction

The measure of the biochemical similarity among organisms is of interest to all persons doing research whose ultimate goal is to determine the essential nature of organisms. A natural consequence of studies in this direction is the systematic classification of organisms based on the similarities and differences in their serological behavior. The blood sera and the soluble protein constituents have been the chief substrates through which serological classifications have been made. Extractions of whole organisms such as insects have also proved to be adequate for comparing the similarity among insect species (Leone, 1947). Systematic serology does not necessarily answer questions on the phylogenetic origin of organisms, but rather associates animals as they exist today into natural classifications based on their essential biochemical similarities.

The following paper is designed to show the type of serological results that can be expected when various families in the insect order Orthoptera are examined serologically. The organisms used in these tests are listed below:

Family	Scientific name	Common name
	Melanoplus femur-rubrum DeG.	Red-legged grasshopper
	Melanoplus differentialis Thos.	Differential grasshopper
	Romalea microptera Beauv.	Florida lubber grasshopper
Acrididae	*Paroxya atlantica* Scudd.	Atlantic locust
	Leptysma marginicollis Serv.	Slender locust
	Spharagemon bolli Scudd.	Boll's locust
	Arphia xanthoptera Burm.	Yellow-winged locust
Tettigoniidae	*Conocephalus strictus* Scudd.	Straight-lanced grasshopper
	Conocephalus fasciatus DeG.	Slender-meadow grasshopper
Mantidae	*Paratenodera sinensis* Sauss.	Chinese mantis
Gryllidae	*Gryllus assimilis* Fabr.	Field cricket
Blattidae	*Periplaneta americana* (Linn.)	American cockroach

Materials and Methods

Preparation of antigens

After first removing the tarsi and the wings the insects were ground in a mortar with sand. Sufficient buffered 0.85 per cent NaCl was added to prevent drying and denaturation of the saline soluble components. The resulting emulsions were cleared using the same techniques described in a previous paper by the author (Leone, 1947). Micro-Kjeldahl nitrogen determinations were performed to determine the total nitrogen and the non-protein nitrogen. Values for the protein content of the antigens were determined as follows: grams total nitrogen − grams non-protein nitrogen = grams protein nitrogen $\times$ 6.25 $\times$ 100 = grams per cent pro-

tein in solution. These values are given in Table I. When only a few insects of a given species were available, it was necessary to dilute the extract sufficiently to obtain volumes large enough to conduct the projected tests. As a result of this the concentrations of protein in solution were markedly reduced in some antigen extracts.

TABLE I

Protein concentrations in extracts of Orthoptera

Organism	Key	Grams protein per cent
Melanoplus femur-rubrum	GR	0.32
Melanoplus differentialis	GD	0.22
Romalea microptera	GF	0.07
Paroxya atlantica	GA	0.05
Leptysma marginicollis	GS	0.03
Spharagemon bolli	GB	0.10
Arphia xanthoptera	GY	0.06
Conocephalus strictus	GL	0.04
Conocephalus fasciatus	GM	0.05
Paratenodera sinensis	MC	0.09
Gryllus assimilis	KC	0.13
Periplaneta americana	CA	0.82

Preparations of antisera

A series of four doubling doses of antigen were injected intravenously in rabbits on alternate days; initial injection was one ml. A second series of antigen injections were given to a rabbit if a preliminary bleeding and testing seven days after the last injection revealed little or no response to the antigen. Final bleedings by cardiac puncture were performed on the eighth day after the last injection. The blood was permitted to clot for 24 hours. The expressed sera were centrifuged, sterile filtered through a Seitz filter, bottled in serum vials, and stored in the refrigerator.

Methods of testing

Ring tests were performed in accordance with standardized procedures as outlined by Boyden (1926).

Photoelectric measurements of precipitin turbidities were made using the Libby photronreflectometer (photron'er) (1938). Titrations were performed and results plotted using the technique of Boyden and DeFalco (1943).

EXPERIMENTAL RESULTS

The production of immune sera against all the Orthoptera except the Blattidae (roaches) proved to be difficult. Protein concentrations were low, and this fact, together with the small quantities of material available restricted the number of injections that could be used to produce an antiserum, and also restricted the number of tests that could be carried out with any one antigen. The photron'er curves proved to have low peaks and broad bases. Inter-family relationships could be established with these curves. Ring test reactions, in general, confirmed the whole curve comparisons of the insect antigens, in so far as this limited technique can be

compared with the nephelometric photron'er method. Typical sets of data are presented to show the correspondence obtained by the two methods (Figs. 1 and 2). A summary of all the information obtained using both techniques is presented in Table II.

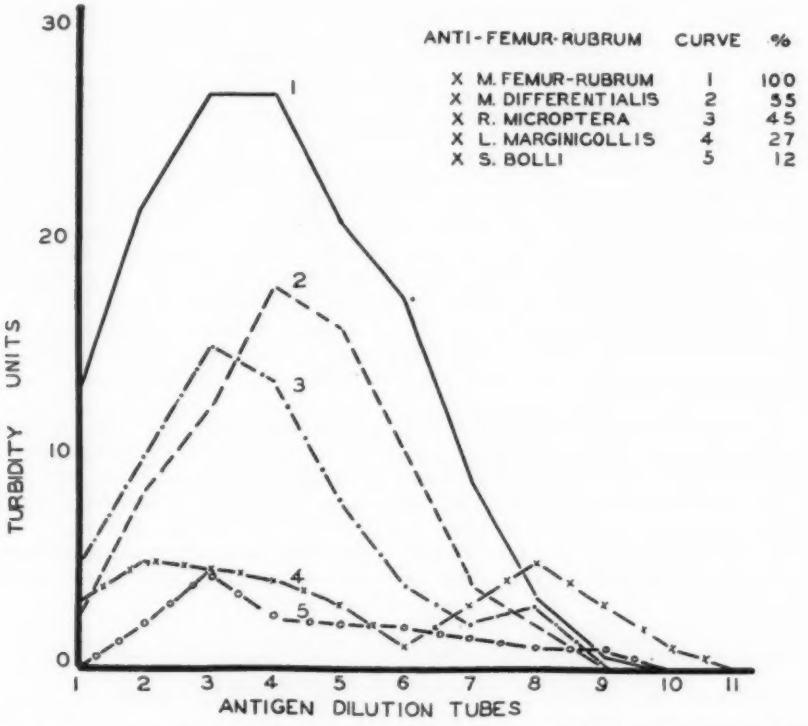


FIGURE 1. A photron'er curve series titrated against an anti-*Melanoplus femur-rubrum* serum. Percentage values indicate the relative degrees of correspondence to the homologous antigen *Melanoplus femur-rubrum*, demonstrated by the various heterologous antigens. The undiluted antiserum shows a low order of turbidities but a broad reactivity range.

Family Acrididae (Short-horned grasshoppers)

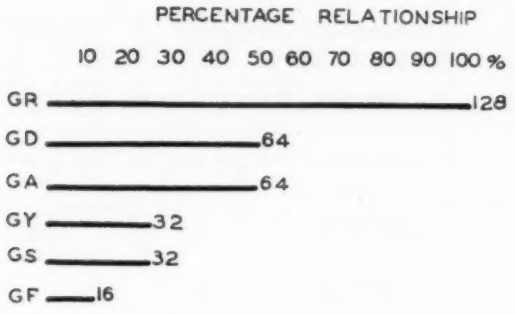
Antisera sufficiently powerful to be recorded on the photron'er were secured in only two instances, one against the Florida Lubber grasshopper (*Romalea microptera*), and the other against the Red-legged grasshopper (*Melanoplus femur-rubrum*). The latter antiserum was obtained as the result of a double series of injections separated by a period of eight days between the first and second injection series.

The anti-Lubber grasshopper serum showed only a slight discrimination between the Red-legged and the Differential grasshoppers (*Melanoplus differentialis*).

TABLE II

Comparison of ring test and photron'er test results

	Antisera	Homologous titer in thousands	Antigens (Relationship values in per cent)											
			Red-legged grasshopper	Differential grasshopper	Florida lubber grasshopper	Atlantic locust	Boll's locust	Yellow-winged locust	Slender locust	Slender-meadow grasshopper	Straight-laced grasshopper	Chinese mantis	Field cricket	American roach
Photron'er Tests	Red-legged grasshopper		100.0	54.9	50.7	44.7		29.2	23.8		12.4	0.0	0.0	0.0
	Florida lubber grasshopper		13.5	9.6	100.0							0.0	0.0	0.0
	Chinese mantis		0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	100.0	6.2	18.7
	Field cricket		6.0	4.5			7.3		2.8			10.5	100.0	29.0
	American roach		0.0	0.0				0.0		0.0	0.0	0.0	4.3	100.0
Ring Tests	Red-legged grasshopper	256	100.0	50.0	25.0	50.0		50.0	50.0		12.5	6.8	12.5	6.8
	Differential grasshopper	32	100.0	100.0		50.0								
	Florida lubber grasshopper	64	12.5	3.4	100.0	0.0	0.0	0.0		0.0		0.0	0.0	0.0
	Atlantic locust	32	100.0			100.0	0.0	0.0	0.0			0.0	0.0	0.0
	Chinese mantis	256	0.0	0.0		0.0	0.0	0.0	0.0		25.0	100.0	25.0	25.0
	Field cricket	256	6.8		6.8	0.0	0.0	0.0	0.0		12.5	6.8	100.0	25.0
	American roach	256	6.8	6.8		0.0	0.0	0.0	0.0		12.5	6.8	25.0	100.0
	Red-legged grasshopper (Diluted 1+1)	128	100.0	50.0	12.5	50.0		25.0	25.0		0.0	0.0	0.0	0.0



Key	*Antigens*
GR	Red-legged grasshopper
GD	Differential grasshopper
GA	Atlantic locust
GY	Yellow-winged locust
GS	Slender locust
GL	Straight-lanced grasshopper
GF	Florida lubber grasshopper

FIGURE 2. A typical plot of ring test titers obtained by heterologous antigens titrated against an anti-*Melanoplus femur-rubrum* serum. The number behind each line represents the highest dilution, in thousands, at which a definite interfacial ring was obtained. Ring tests performed using undiluted antiserum gave undifferentiating titers among all the *Acrididae* except the Lubber grasshopper. A dilution of the antiserum with equal parts of saline gave the above discrimination.

The anti-Red-legged grasshopper serum demonstrated an ability to react against many heterologous antigens. Even closely related forms could be readily distinguished, however of the species that showed reactivity, the Straight-lanced grasshopper (*Conocephalus strictus*) belonging to the more distant Tettigoniidae, showed the least amount of turbidity. The mantids, crickets, and roaches produced no heterologous turbidities in the photron'er and thus indicated their more distant relationship.

The confirmation of the reduced reactivity of these more distant species when tested with the ring test was striking. Intra-family differences could not be demonstrated with the ring test (exception may be cited in the case of the Lubber grasshopper *Romalea microptera*). A dilution of one part serum to one part saline reduced the reactivity of the antiserum to such an extent (and also increased its specificity) that all organisms outside the family no longer reacted.

Antisera procured against other members of the family Acrididae, i.e., the Differential grasshopper and the Atlantic locust were too weak to give photron'er readings. Ring test titers for these organisms were very low also, the homologous reactions having a titer of only 1 to 32000.

Family Mantidae (Praying mantids)

Only a single species of this family, the chinese mantis (*Paratenodera sinensis*) was secured. The photron'er results indicate a very specific antiserum with the

families Blattidae and Gryllidae barely making their appearance. The Blattidae (*Periplaneta americana*) show a closer relationship to the Mantidae than any other family considered.

Ring test results indicate undifferentiating heterologous titers among the cricket, roach, and Straight-lanced grasshopper, representing their respective families Gryllidae, Blattidae, and Tettigoniidae when compared against the Mantid. The family Acrididae, evidently more distant, did not react.

Family Gryllidae (Crickets)

In view of the morphological indistinctness of the several large black species of crickets, all the specimens collected were pooled and treated as a single type species (*Gryllus assimilis*) to represent this family. By photron'er test the *Blattidae* (roaches) showed the closest relationship; other families, on the whole, reacted weakly. Greater differentiation of these distant relatives was shown by the ring tests.

Family Tettigoniidae (Long-horned grasshoppers and katydids)

Two species were secured to represent this family, the Straight-lanced grasshopper (*Conocephalus strictus*) and the Slender-meadow grasshopper (*Conocephalus fasciatus*). Antisera were not obtained against these antigens. Both species were used however in the heterologous reactions to aid in establishing their position with respect to other species and the position of other species with respect to them.

Family Blattidae (Roaches)

One sample of the American cockroach (*Periplaneta americana*) was chosen to represent this family. The antiserum was the most powerful of all those tested, i.e., gave the greatest turbidity readings, but also was among the most specific of the antisera with respect to inter-family reactions on the photron'er. The field encompassed by means of the ring test technique is somewhat broader than the photron'er examination of this antiserum. The Gryllidae reactions were the most nearly like the Blattidae, of all the families tested. The Tettigoniidae, Acrididae, and the Mantidae showed lessened but approximately the same degrees of relationship to the Blattidae.

Discussion

The results as presented above are at best only a beginning in the study of the problem of the quantitative systematic serology of insects. The insect species used in the tests were chosen because of their availability to the writer. No attempt was made to choose species in such a way, or to perform tests in such a manner as to solve particularly significant problems in insect relationships. It was desired to know the type of serological results that could be obtained using extracts of whole insects as antigens characteristic for the species tested.

The results do indicate the feasibility of making a quantitative serologic analysis of representatives of the families, genera, and species of insects.

Boyden (1943) has been able to establish relatively constant inter-species, intergeneric, and inter-family relationships among the Crustacea. His average value for

the relationship of sera of the same genus is 46 per cent; the averaged value for relationships of genera in the same family is 30 per cent; and, for inter-family relationships the average is 9 per cent.

Intra-generic values for the two species of Melanoplus considered in this paper have a value of 54.7 per cent. The averaged relationships of extracts of genera within the insect family Acrididae proved to have a value of 28 per cent. Inter-family relationships for the order Orthoptera have an average value of 11.5 per cent. The extremes of the serological relationship values for any given group using extracts of fresh insects are all within the extreme values reported by Boyden, who used whole sera for his comparisons. The averaged values obtained for relationships between species, genera, and families are in accord with the Crustacea values.

More information will have to be acquired on the nature of serological reactions, and more data accumulated on actual systematic tests before serological limits in terms of percentages, or other statistical rankings, can be summarized or defined as representing "species," or "genera," or "families" of insects.

Tentative serological relationships for four of the five families of Orthoptera investigated are given in Figure 3. The fifth family tested, the Tettigoniidae are not

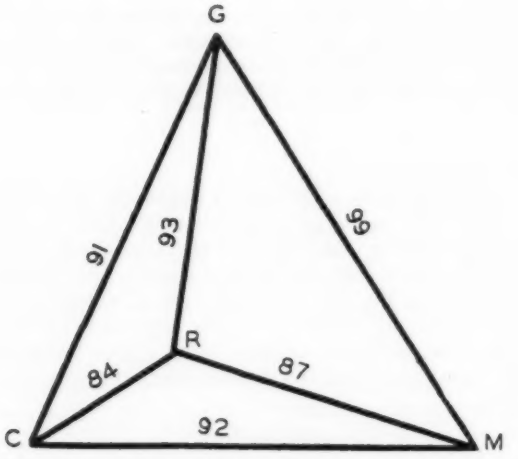


FIGURE 3. A diagram to show the relative distances of four families of Orthoptera from each other. The data are tentative inasmuch as the families have not yet had really adequate testing. The families concerned are Blattidae (*R*), Mantidae (*M*), Gryllidae (*C*), and Acrididae (*G*). The figure requires three dimensions for adequate representation, hence values contiguous to the Blattidae locus are not in proportion with the remainder of the figure. A value of 99 represents the maximum measurable distance between any two families.

represented in Figure 3 inasmuch as no antiserum was produced against this family and reciprocal relationships could not be established. It should be pointed out that the values given are not absolute but only relative. A summary of the relationships of these Orthoptera as determined by the comparison of extracts made from whole insects is as follows:

A. Blattidae—approximately equidistant from the Mantidae and Gryllidae and closer to these families than to the Acrididae.
B. Mantidae—most closely related to the Blattidae. Approximately equally related to the more distant Gryllidae and Acrididae.
C. Gryllidae—closer to Blattidae than to Mantidae and Acrididae.
D. Acrididae—appears to be more closely related to Gryllidae and Blattidae, than to the Mantidae.
E. Tettigoniidae—most distant from the Mantidae and Blattidae of all the families tested. Shows slightly more relationship to the Acrididae than to the other families.

There is striking correlation between the findings of Crampton (1932) in his taxonomic and phylogenetic studies and the orientation of insect families as discussed above, indicating that there may be some correlation between time of origin and degree of similarity in the antigenic constituents of insects, i.e., their serum proteins and other extractable proteins. Except for the position assigned to the Gryllidae, there is also general agreement between the phylogenetic tree of Walker (1922) and the relative positions occupied by the five families of Orthoptera investigated in this paper. As stated previously, however, systematic serology does not attempt to give a final answer to questions on the phylogenetic origin of organisms, but rather associates animals as they exist today into natural classifications based on their essential biochemical similarities. Where serological evidence is in agreement with a paleologic-taxonomic study which correlates phylogenetic origin and present day classifications, then the biochemical (i.e., serological) evidence becomes additive to the other two and increases the likelihood that such a study presents the true picture of the evolutionary development of the species examined.

Summary

1. Representatives of five families of the insect order Orthoptera were compared serologically.

2. On the basis of the degree of serological similarity among them the relative positions of these five families are given.

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THE ACTION OF ELECTRICAL STIMULATION AND OF CERTAIN DRUGS ON CARDIAC NERVES OF THE CRAB, CANCER IRRORATUS

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Introduction

The nervous regulation of the decapod crustacean heart has been studied for over a century, yet the nature of the mechanisms involved are still obscure. The nerves concerned are minute, and only in recent years has a picture been obtained of the anatomical relationships of the cardio-regulatory nerves to the heart and its ganglion cells.

Inhibitory cardiac nerves in crayfish were early indicated or demonstrated by several investigators including Dogiel (1876, 1877), Yung (1878), and Plateau (1878, 1880), whose work showed in a general way that these nerves arise from the anterior part of the thoracic ganglionic chain or mass. Dogiel believed that nerves arising anterior to the sternal artery and running to the pericardium and abdominal extensor muscles were inhibitory, but as his only points of electrical stimulation were the pericardium and the thoracic nerve cord between the bases of the second and third leg nerves it does not appear that he traced the inhibitory nerves in detail. In view of frequent misquotations it is well to note that "Dogiel's nerve" was never claimed to run along the sternal artery or actually to enter the heart. Inhibitory cardiac nerves in the crayfish have recently been traced by Wiersma and Novitski (1942).

Definitive information on the course of inhibitory cardiac nerves in crabs rests upon the work of Jolyet and Viallanes (1892, 1893), Conant and Clark (1896), and Bottazzi (1901). Conant and Clark demonstrated most clearly in *Callinectes* that the inhibitory nerves arise as a single pair close to the bases of the recurrent cutaneous nerves, and run with these large nerves anterodorsally from the thoracic ganglion, eventually separating and joining the cardio-accelerator nerves on each side to form the lateral pericardial plexus. The entrance of the inhibitory nerves into the heart was not observed by Conant and Clark, but the observations of Alexandrowicz (1932), Heath (1941), and the writer are in agreement that the cardio-regulatory nerves enter the crab heart as a single dorsolateral pair of delicate strands, containing very few fibers.

Interest in cardio-accelerator nerves at first centered about "Lemoine's nerve," which was described in the crayfish by Lemoine (1868) as arising from the stomatogastric nervous system on the anterior dorsal wall of the stomach. This nerve, which is extremely fine, was reported to run beneath the ophthalmic artery and to break up as it entered the heart. In view of later misquotations, it is well to note

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that Lemoine did not derive his cardiac nerve from the cerebral ganglia, nor did he produce any effects on the heart by stimulation of the "brain." The positive results of stimulation of "Lemoine's nerve" reported by several authors (Lemoine, 1868; Yung, 1878; Plateau, 1880) were possibly a reflex phenomenon. More recent workers, including Jolyet and Viallanes (1892, 1893), Conant and Clark (1896), Wiersma and Novitski (1942), have not found "Lemoine's nerve" to be a cardio-accelerator. Vital staining studies by Alexandrowicz (1932), Heath (1941) and the writer show that this nerve in the crab innervates the anterior valves of the heart, and does not pass to heart ganglion or muscle. Jolyet and Viallanes (1892, 1893), confirmed by Conant and Clark (1896), showed that the cardio-acceleratory nerves in crabs consist of two pairs arising dorsal to the bases of the nerves to the third maxilliped and the first leg. The two acceleratory nerves on each side join the single inhibitory nerve to form the lateral pericardial plexus, from which the acceleratory nerves pass into the heart in the same thin strand which carries the inhibitors.

The cardiac ganglion (intrinsic ganglion) of the crab heart, as in other marine decapods, contains about nine cells and sends its fibers in a complex but regular pattern throughout the heart (Alexandrowicz, 1932). It is innervated by the single pair of nerves carrying inhibitory and excitatory fibers. The exact relationship of each type of fiber to the heart ganglion and heart muscle is not fully understood. Alexandrowicz and others have observed that the fibers entering the heart are of two sizes, the larger of which make synaptic contact with processes of the ganglion cells, while the smaller fibers pass to the muscle. Alexandrowicz suggests that the thicker fibers are inhibitory, the thinner excitatory. However, in their studies of the multiple innervation of crustacean limb muscles, van Harreveld and Wiersma (1939) have found that the inhibitory fiber is thinner than the motor fibers to the same muscle, while Wiersma and Novitski (1942) have shown that the cardio-acceleratory nerves in the crayfish probably act upon the heart ganglion rather than on muscle.

Pharmacological studies have indicated that a cholinergic nervous mechanism is concerned in the initiation of heartbeat in higher crustaceans (Welsh, 1939a, 1939b; Davenport, Loomis, and Opler, 1940; Davenport, 1941, 1942; Prosser, 1942 and others). The isolated decapod heart is stimulated by acetylcholine, an effect augmented by eserine and blocked by atropine. While it is generally agreed that the intrinsic ganglion has a pacemaker function, and that it is stimulated by acetylcholine (Welsh, 1942), attempts to determine whether acetylcholine acts in a muscarine-like or in a nicotine-like fashion on the ganglion or muscle of the isolated heart (Davenport, 1941, 1942) have revealed little concerning the manner of action of the regulatory nerves which reach the heart from the central nervous system.

In pharmacological studies of the isolated heart it has generally not been possible to tell at what sites the drugs used are actually taking effect. For instance, the rate of the isolated crustacean heart is quickened by acetylcholine. This effect is probably due to the action of the drug on the heart ganglion, but the possibility of a direct action on the muscle remains. The sensitivity of the ganglion to acetylcholine does not prove that the neurons of the ganglion are themselves cholinergic. They might be cholinergic, adrenergic, or of some still unknown nature. It is helpful to recall that in vertebrates cholinergic preganglionic fibers may stimulate either

cholinergic or adrenergic postganglionic neurons in the autonomic nervous system. Thus the situation in the isolated crustacean heart is more complex than it may appear at first glance. When a drug is applied to the whole heart, it undoubtedly has some effect on muscle as well as on the ganglion, and if we admit the possibility of its stimulating the remnants of regulatory nerve still within the heart, we see further complications, not only because we may not be able to identify the effects of the regulatory nerves in the total response, but also because the simultaneous stimulation of these opposing nerves could produce conflicting effects. Since the completion of the work reported here, Wiersma and Novitski (1942) have shown that perfused acetylcholine and excitatory nerve stimulation produce similar effects in the crayfish heart, and that eserine augments the effect of nerve stimulation upon heart rate. These observations may be considered as excellent evidence for the cholingeric nature of the cardio-accelerator nerves and for the termination of these nerves upon the ganglion cells rather than on muscle. However, it is still not known at what point in the heart the inhibitory nerves act. Previous studies by the writer on nervous inhibition of the heart of *Panulirus* (Smith, 1940–41) led to the conclusion that the cardiac ganglion was possibly cholinergic, but that the inhibitory mechanism was not of the "muscarine-like" cholinergic type.[2]

In view of the scarcity of direct evidence on the nature of the regulatory heart nerves it was felt that further study of the effects of certain drugs on the action of these nerves in a semi-intact preparation might yield information regarding their nature and mode of action. It was originally hoped that this work could include an electrical study of the heart ganglion when the heart was being inhibited or excited by nervous action, in order to clarify the role of the heart ganglion in nervous control, but, unfortunately, exigencies of 1942 did not permit electrical studies to be made.

Throughout this work I received constant encouragement and much helpful criticism from Dr. John H. Welsh, to whom I wish to express my gratitude.

Material and Methods

The eastern rock crab, *Cancer irroratus,* was found to be of suitable size and hardiness for winter laboratory use if maintained under cold conditions. By suitable cannulation, the heart was perfused *in situ* with physiological solutions or drugs. Heart action was recorded kymographically, while stimulation was applied to the inhibitory and to the excitatory nerves by means of two sets of mechanically manipulated electrodes which were left in place upon the nerves through the course of an experiment. Exposure of the thoracic nerve mass from beneath gave access to these nerves close to their point of origin, at a considerable distance from the heart. Stimulation was supplied either by a pair of Harvard inductoria, or by a thyratron stimulator (modified after Delaunois, 1939) giving repetitive shocks at controllable frequencies and voltages.

Perfusion fluid was made up following Cole's (1940) analysis of the blood of *Cancer borealis.* (An analysis of the blood of *C. irroratus,* generously carried out by Dr. Cole, showed that the composition of the blood of this species is similar to to that of *C. borealis.*)

[2] The statement in the original report (Smith, 1940–41) that, "the inhibitory mechanism is not of the cholinergic type," was not justified by experimental results, and should be in the less sweeping form, "not of the muscarine-like cholinergic type."

NaCl	0.506 M	1000 parts			
KCl	0.506 M	26.1 parts	$MgCl_2$	0.506 M	8.7 parts
$CaCl_2$	0.506 M	26.1 parts	$MgSO_4$	0.506 M	39.1 parts

To each liter of solution was added 17.6 cc. of 0.5 M boric acid and 0.96 cc. of 0.5 M NaOH as a buffer.

The crab heart perfused *in situ* exhibits a steady beat which may be slow or rapid, depending upon the temperature and the conditions of the dissection and perfusion. For best results, all the supporting ligaments of the heart should be intact, although a fairly good beat may often be obtained when the posterodorsal ligament has been severed. A low temperature (10° – 15° C.) is much more favorable than room temperature at any season. The pressure within the heart, which depends upon the rate of perfusion as well as upon the integrity of the heart and its valves, largely controls the amplitude and rate of beat. In diastole the heart is stretched horizontally by its elastic supporting ligaments until it presents a broad, flat or slightly depressed dorsal surface. In systole, the internal pressure causes the dorsal surface to bulge upwards as the heart passes from a flat to a more rounded cross-section, while at the same time, the rear wall of the heart bulges posteriorly. This latter motion was recorded in the tracings as a sharp upward deflection of the writing lever.

With insufficient internal pressure, the heartbeat is weak and irregular, or may even cease. Too great pressure causes a marked increase in amplitude and rate of beat. In this work, the perfusion rate was adjusted until the heart was beating steadily, and not showing more than slight passive swelling between beats. Sudden increases in the rate of perfusion produce an increase in the amplitude and rate of beat strikingly similar to that caused by the excitatory nerves. That this is not a reflex phenomenon involving these nerves can be shown by its continuance after complete removal of the central nervous system. On the other hand, a decrease in the pressure exerted within the heart by the perfusion fluid may cause slowing or cessation of beat. These results of internal pressure changes will be found helpful in explaining certain after-effects of nervous excitation and inhibition.

Effects of Faradic Stimulation of Cardio-Regulatory Nerves

Nervous inhibition

The inhibitory nerve to the heart was stimulated close to the basal portion of the recurrent cutaneous nerve, just forward of the ventral thoracic ganglion (see Fig. 1). With the ventral approach to the inhibitory nerves, there is little danger of confusion from accidental stimulation of the excitatory nerves. In this work, stimuli only slightly above threshold have been used, and in properly set up preparations no effects of any spread of current from the point of stimulation have been noted.

With inductorium stimulation, cardiac inhibition is usually complete, there being only a very narrow range in which partial inhibition can be obtained. For this reason, the inductorium is useful mainly to determine the intensity threshold for complete inhibition. The inhibition obtained by these means is an abrupt cessation of heartbeat when the intensity threshold is reached. This persists for ten to sixty seconds if the stimulus is continued, after which "escape" beats occur, leading to restoration of a normal beat. If the stimulus is discontinued while the heart is stopped, the beat is restored immediately, without perceptible inhibitory after-

effect. In a few cases, the first beats after inhibition are of increased amplitude or rate. Observation has shown that during the period of inhibition, the continued inflow of fluid sometimes stretches the heart passively, as indicated by a rise in the traced record. When a stretching of the muscle occurs, the effect at the restoration of beat is the same as that resulting from an increase in perfusion rate, hence the heart responds by an augmentation of beat. In cases where the rate of perfusion is low, or when the heart stopped in diastole still tends to drain naturally, there is no evidence of distention, and as a result, no stimulatory after-effect of inhibition.

Nervous excitation

The two pairs [3] of excitatory nerves arise dorsal to the bases of the nerves to the third maxillipeds and the chelipeds respectively (see Fig. 1). Stimulation of these nerves in the vicinity of the heart is not effective because of interference from the nearby inhibitory fibers, but they are easily stimulated as they leave the thoracic ganglion. Commonly, the electrodes were placed to the rear of either of the large nerves mentioned above, dorsal to whose roots the heart nerves arise. However, because of the fact that the inhibitory nerves arise only slightly anterior to the excitatory, it is advisable to sever the inhibitory nerve on the side where the excitatory nerves are being stimulated. Excitation caused by faradic stimulation does not show a sharp threshold. It develops gradually with increasing intensity of stimulation, and soon reaches a maximum. One cannot well speak of "partial excitation." It would have been desirable to establish a quantitative measure of the amount of excitation produced, but because of the great variation in the character of the response in different hearts no reliable method was devised. According to the condition of the valves after the manipulation attending cannulation, the accelerated heart may literally pump itself dry, or it may retain fluid and increase the internal pressure. With these and possibly other variables operating to modify the picture of nervous augmentation of heartbeat, faradic stimulation of the excitatory nerves may produce an increase either in rate or amplitude, or in both. The after-effect of excitation is likewise variable, but in contrast to inhibition, excitation commonly shows a stimulatory after-effect, which in some cases lasts for several minutes after stimulation has ceased. This is most noticeable when a preparation is fresh.

A factor which may act to conceal a stimulatory after-effect, and which may even result in a depression, is the tendency of some hearts to pump themselves dry when accelerated, leading to temporary cessation of beat following the period of excitatory stimulation. Nevertheless, the number of times that excitation of the heart has resulted in an unquestionable after-effect in these experiments, as well as in those of earlier workers (Bottazzi, 1901; Conant and Clark, 1896), makes it appear that this phenomenon is a characteristic feature of nervous excitation of the crab heart, as a result of faradic stimulation.

That there is an upper limit to the effectiveness of the excitatory nerves is shown by their inability to produce a state of tetanic contraction in the heart, although the heart may readily be tetanized by direct stimulation, while acetylcholine

[3] No excitation of the heart by stimulation of "Lemoine's nerve" has been noted in this work.

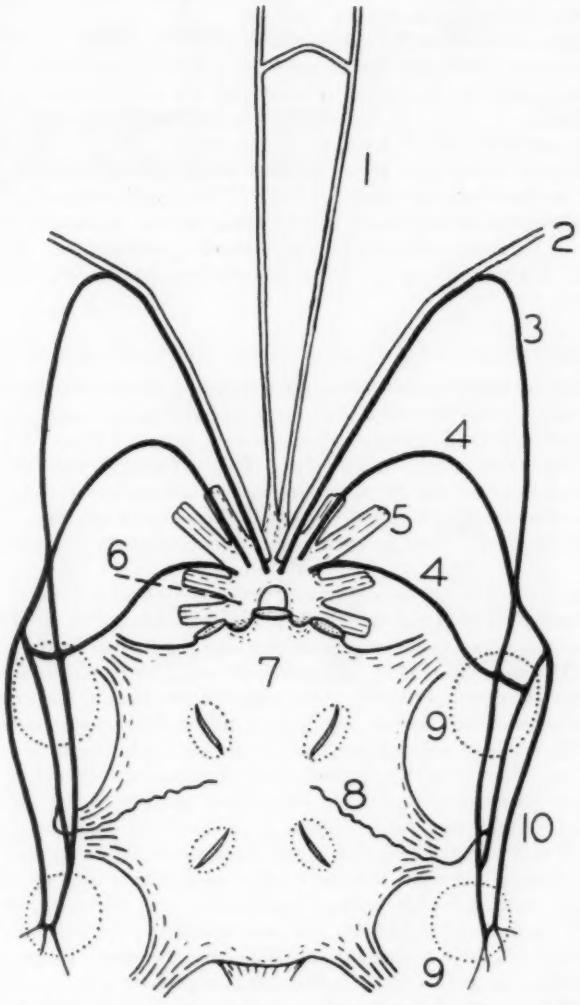

FIGURE 1. Schematic representation of the innervation of the heart of *Cancer* in dorsal aspect, with the thoracic ganglion shown as if moved forward slightly. Approximately to scale, but with cardiac nerves and pericardial plexus drawn with disproportionately heavy lines. (*1*) Circumoesophageal connective, (*2*) recurrent cutaneous nerve, (*3*) inhibitory cardiac nerve, (*4*) (*4*) excitatory cardiac nerves, (*5*) nerve to cheliped, (*6*) thoracic nerve mass, (*7*) heart, (*8*) dorsal nerve entering heart, (*9*) (*9*) branchio-cardiac orifices, (*10*) lateral pericardial plexus.

in high concentrations has been shown to cause an accelerated beat leading to a tetanus (Welsh, 1939a, b; Davenport, 1941).

Such results indicate that the extrinsic excitatory nerves do not directly control the rate of heartbeat, but must act to alter the rate of spontaneous activity in the pacemaker mechanism of the heart. Presumably the effectiveness of these nerves lies in their ability to alter the conditions determining the activity of the cardiac ganglion without being able to force the latter beyond certain limits.

It is commonly stated that the crustacean heart muscle, unlike the vertebrate heart, shows no absolute refractory period. This generalization holds when the heart is stimulated via the excitatory nerves, since cardiac excitation may commence at any phase of the beat, resulting in a well-marked summation. In this respect, the crustacean heart reacts rather more like skeletal muscle than like vertebrate cardiac muscle.

Effects of Frequency of Stimulation Upon the Cardio-Regulatory Nerves

In an effort to obtain a clearer picture of the action of the cardio-regulatory nerves, they were stimulated over a wide range of frequencies, employing a thyratron stimulator which delivered repetitive shocks over a range of 10 to 1,800 per second, with voltage adjustable from 0 to 10 volts. This stimulator could be connected at will to either of the two sets of electrodes in use, allowing comparable studies to be made on excitatory and inhibitory nerves in the same preparation over the same period. Both electrodes were fixed in place and undisturbed through an experiment.

Preliminary experiments showed that at very low or high frequencies the voltage required to produce a response was higher than at intermediate frequencies. Accordingly, a series of trials was made to determine the threshold voltage at various frequencies. Frequency-intensity threshold curves were plotted, showing the changes in intensity threshold over a wide range of stimulation frequencies, and the frequency limits above and below which stimulation of the heart nerves produced no effect. The results of a typical experiment are shown in Figure 2.

The greatest difficulty in this method is the problem of recognizing the onset of response in the heart at low or high frequencies outside of the optimum range. Over most of the frequency range, inhibition is total and begins abruptly, so that the threshold is easily determined, but at low frequencies, inhibition may first appear as a slight, gradually increased slowing of the heart, which may or may not cease beating abruptly when a higher voltage is reached. In such cases, two thresholds must be noted, one for partial, and one for total inhibition. In the case of excitation, while the onset of response is sharp over most of the frequency range, there may be a gradual acceleration at low frequencies that is especially hard to detect. It has not been possible to establish any criterion of "partial" excitation, hence the first noticeable increase in rate or amplitude has been taken as the threshold. Obviously, in all tests of this sort it is important to raise the intensity at a uniform rate, ceasing to raise the intensity as soon as results are observed on the kymograph tracing. Further to lessen the subjectivity of observations, tests were carried out in a planned series without stopping to verify individual readings. The series of frequencies were then passed through in the reverse direction in such a way that descending frequency settings alternated with those of the ascending series. No curves were con-

INTENSITY —— VOLTS

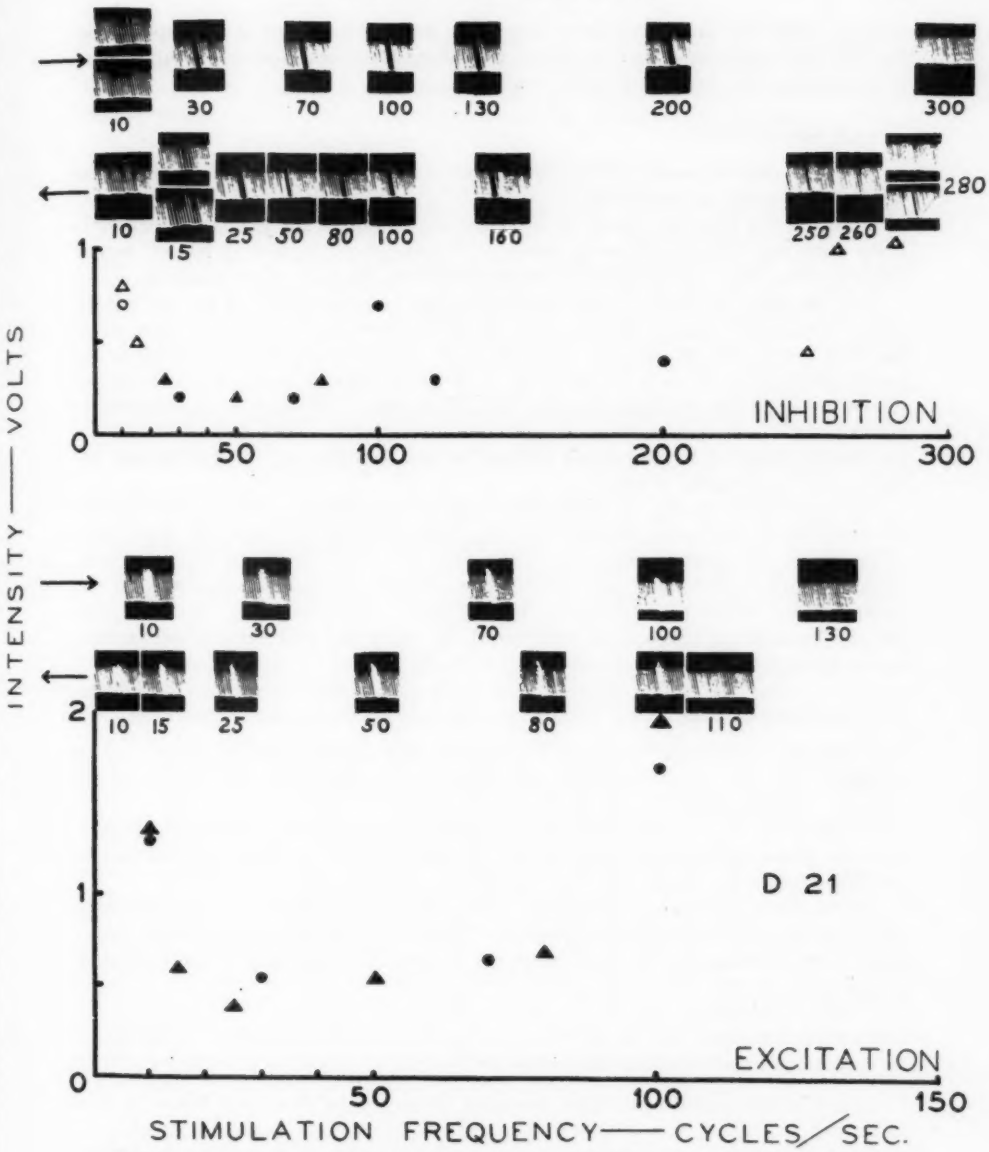

FIGURE 2. Frequency-intensity threshold curves for stimulation of the cardiac inhibitory and excitatory nerves, with tracing of the heart response at each frequency. Circles on the curve represent determinations made on an ascending scale of frequencies; triangles those on a following descending frequency series. Both curves obtained from the same animal (D–21), with tests made alternately on inhibitory and excitatory nerves. Open symbols indicate thresholds for partial inhibition at frequencies where no total inhibition could be obtained.

sidered valid if the electrodes had to be moved during the course of the experiment. Certain generalizations may be made regarding the effects of the frequency of stimulation on the cardio-regulatory nerves.

The excitatory nerves

1. The excitatory nerves show a low intensity (voltage) threshold for stimulation over a wide range of frequencies.

2. The lower frequency limit for effective stimulation of the excitatory nerves is lower than that for the inhibitory nerves. At frequencies as low as 10 per second there is often only a very slight rise in the threshold intensity of stimulation.

3. The upper frequency limit of effectiveness of stimulation of the excitatory nerves is variable, usually between 100 and 300 per second, but in some cases ranging well above 300 per second. In such cases it is unlikely that the nerve is actually discharging at such a high rate. The usual upper frequency limits are in agreement with the findings of Bogue and Rosenberg (1936), who report that in the leg nerves of *Maia* a large proportion of the motor fibers respond regularly to stimuli delivered at the rate of 200 per second, but that at 400 per second the fibers cease to respond in phase.

4. In the lower range of stimulation frequencies, higher voltages are required to stimulate the excitatory nerves, and less effect is produced upon the heart than when higher stimulation frequencies are employed. Raising the intensity of low-frequency stimulation does not produce the marked increase in response of the heart that can be produced by raising the frequency. This finding is consistent with the small number of nerve fibers innervating the heart and with the marked facilitation exhibited by crustacean nerves.

The inhibitory nerves

1. There is a frequency range from about 15–20 stimulating shocks per second up to 100 or 200 per second in which inhibition is complete at or near the threshold voltage of inhibitory nerve stimulation.

2. At a lower range of frequency, inhibition becomes only partially effective. In some preparations, stimulation of the inhibitory nerve at 10 per second has no visible effect on the heart, regardless of the stimulus intensity.

3. At the upper range of effective frequencies, stimulation of the inhibitory nerve often causes only partial inhibition. There is so much variation in the upper limit of effectiveness that no significance can be attached to its value in the individual preparation.

The effect of aging of the preparation upon the frequency-intensity threshold curves for inhibitory and excitatory nerves

Since in the experiments with perfused drugs, to be described below, it was necessary to run repeated series over periods of several hours, a number of undrugged preparations were tested repeatedly in order to determine the effects of fatigue. There is exhibited in successive runs a slight raising of the intensity threshold, associated with a narrowing of the frequency range over which stimulation of the heart nerves is effective. This is most marked at the upper end of the

frequency range, where no certain significance can be attached to it. The lower end of the frequency curve shows much less tendency to shift with aging than does the upper. For this reason, in the experiments with drugs, the main attention was directed to that portion of the frequency curve lying below 100 per second.

Simultaneous Stimulation of Inhibitory and Excitatory Nerves

Using two inductoria, excitatory nerves have been stimulated during the course of nervous inhibition. In all cases where equal intensities have been used, inhibition remains dominant, and is not interrupted even when much stronger intensities of stimulation are applied to the excitatory nerve. Also, if an inhibitory nerve is stimulated during a period of nervous excitation, inhibition readily interrupts the excitation. But since a stronger inhibitory stimulus is required to stop a heart already under the influence of the excitatory nerve than is required to stop the unstimulated heart, it would seem that to a certain extent the inhibitory and excitatory nerves are each able to modify the effects produced by the other.

Further work, especially electrical studies of the ganglionic pacemaker within the heart, is needed before it can be said whether or not the "blending" of the actions of inhibitory and excitatory heart nerves means that these opposing nerves affect the same portion of the pacemaker system.

Effects of Strychnine upon Nervous Excitation and Inhibition of the Crab Heart

A number of tests have been made of the action of strychnine upon the cardiac inhibitory and excitatory nerves. Because strychnine in the concentrations necessary to affect the regulatory nerves has been found to affect the heartbeat adversely, about half of the experiments have been inconclusive. This is especially true in regard to studies on inhibition, since a heart that has stopped beating cannot be further inhibited, even though it can frequently be made to beat by stimulation of the excitatory nerves. The effects of strychnine on the character of the heartbeat generally follow a sequence that is passed through more or less quickly depending upon the concentration of drug applied. There is first a moderate increase in rate and amplitude of beat, giving way to a phase of grouped beats, in which groups of 2–8 beats are separated by short (2–5 second) pauses. With a decrease in the number of beats in a group, a state is reached in which the heart records single spike-like beats at intervals of 10–30 seconds. These beats commonly pass to the form of brief tetani, lasting some 1–4 seconds, which are a very characteristic result of strychnine in the crab. If poisoning is more severe or long continued, the heartbeats may become irregular or cease altogether, although retaining their amplitude to the last. The heart will usually recover if thoroughly washed.

The effects of strychnine upon nervous inhibition

Twelve experiments were performed, of which three were discarded because the electrodes had to be moved during the run, indicating poor setting or local injury to the nerve, while in three the heart failed before all the necessary tests could be car-

ried out. The remaining six indicate that strychnine, in concentrations of about 1:2500 (4×10^{-4}), is able to abolish the effect of the inhibitory nerves after perfusion for 30 to 60 minutes. The loss of response was verified in all cases by decreasing the coil distance of the stimulating inductorium by 5 mm. After a short period of washing, during which the beat usually showed a temporary depression, the inhibitory effect of the nerve was restored to or nearly to the initial value; in all cases the threshold after restoration was lower than the strongest stimulus used to verify the abolition of inhibition. In each of two experiments the abolition of inhibition in strychnine and its restoration after washing were repeated four times.

The effects of strychnine upon nervous excitation

The records of the effects of strychnine upon the effectiveness of the excitatory nerves to the heart are somewhat more reliable than are those for inhibition, since excitatory effects may frequently be obtained even after the heart has ceased to beat. Strychnine in concentrations of 1:2500 (4×10^{-4}) blocks the action of the excitatory heart-nerves, and this effect is fully reversible.

These experiments indicate that the cardiac regulatory nerves can be paralyzed by strychnine, as the somatic motor nerves presumably are, without causing the heart to cease beating. However, the anomalous character of the beat in the strychninized heart indicates that the intrinsic pacemaker neurones are affected considerably, though perhaps to a lesser degree than are the regulators.

Effects of Atropine Upon Nervous Inhibition and Excitation

Methods of study involving the use of inductorium stimulation, as in the work on the effects of strychnine described above, failed to reveal any effect of atropine on either the excitatory or the inhibitory nerves. Accordingly, confirmatory studies were made by the method of variable-frequency stimulation. Frequency-intensity threshold curves have been determined before and during perfusion with atropine, as well as after a period of washing.

The effects of atropine upon nervous inhibition

Atropine sulphate was perfused in concentrations of 10^{-5} and 10^{-4} for periods of 15–30 minutes before determining the frequency-intensity curves for the inhibitory nerves while the heart was still being perfused with the drug. The application of the drug followed, of course, the determination of such a curve while the heart was bathed with perfusion fluid. The cardio-inhibitory nerves of *Cancer* are not blocked by atropine, but are actually slightly augmented in their effects.

The effects of atropine upon nervous excitation

Atropine sulphate was perfused in concentrations of 10^{-6} to 10^{-4} for 15–30 minutes, as in the preceding experiments. The frequency-intensity threshold curves indicate that atropine in these concentrations has no significant effect upon the cardiac excitatory nerves. That a sufficient concentration of atropine (10^{-4}) was used is indicated by the fact that Welsh (1939b) found that atropine 10^{-5}

would largely block the effects of acetylcholine on the isolated heart of *Panulirus*, while atropine 10^{-4} is effective in this respect on the heart of *Astacus* (Davenport, Loomis, and Opler, 1940) and atropine 5×10^{-5} on the isolated heart of *Cancer magister* (Davenport, 1941).

Effects of Nicotine Upon Nervous Inhibition and Excitation

In view of the failure to block nervous inhibition and excitation with atropine, it was of interest to see how nicotine might affect these processes, since certain effects of acetylcholine ("nicotine-like" effects) are not abolished by atropine. A number of experiments were performed, using faradic stimulation. The results have been checked by variable-frequency stimulation. Nicotine, when first applied, has a strong augmenting effect on amplitude and rate of beat. The stimulatory effects wear off after a time, and, if the concentration of nicotine is high, depression of heartbeat ensues. Irregularity and grouping of beats are common in the early stages of nicotine depression; later stages are marked by infrequent beats or by an abnormal condition of intermittent tetanus. This last type of beat may be the result of a direct response of the heart muscle to stretching, as it could be seen in the records that the heart swelled noticeably between "beats." The crab heart adapts to nicotine, hence successive increases in the strength of drug applied produce relatively small effects.

The return of the heart to perfusion fluid after being in a strong nicotine solution frequently results in a temporary cessation of beat, following which the beat may be greatly accelerated as the nicotine concentration falls from a depressant to an excitatory level. During the period of complete stoppage, the excitatory nerve is able to function, an indication that this nerve is more resistant to the paralyzing effects of nicotine than is the cardiac ganglion.

The effects of nicotine upon nervous inhibition

In no case has inhibition been abolished by nicotine in concentrations of 1 : 10,000 to 1 : 1,000. Hearts responding to freshly applied nicotine by a marked frequency increase can be inhibited as readily as the normal heart, or as hearts which have been greatly depressed by strong concentrations of nicotine. The action of the inhibitory nerves can be studied only as long as the heart continues to beat, hence the effect of dosages of nicotine of strengths greater than 1 : 1,000 could not be determined.

The effects of nicotine upon nervous excitation

Nicotine in strengths of 1 : 1,000,000 to 1 : 500 has been tested, with inductorium stimulation of the excitatory nerves. The results fall into three classes, depending upon the strength of drug used and its effect on the rate of heartbeat. Nicotine in the lower concentrations (10^{-6}), or when first applied, has a marked positive chronotropic effect which may persist for some time. In certain experiments, stimulation of the excitatory nerve during this period of excitation has shown an augmentation of the effectiveness of the nerve as compared to its effectiveness upon the undrugged heart.

In intermediate concentrations of nicotine (10^{-5}), or in concentrations applied for a length of time sufficient to depress the heartbeat, nervous excitation is not

impaired. This fact indicates that the cardiac ganglion is more sensitive to nicotine depression than are the excitatory nerves. The complete stoppage of heartbeat that may follow the return of a heart to perfusion fluid after being in nicotine supports this view, since the excitatory nerves may stimulate a heart that has ceased its spontaneous beat under such conditions. In this situation the pacemaker ganglion has evidently lost its capacity for spontaneous discharge, although it must still be able to transmit impulses to the heart muscle via the neuromuscular junctions when properly stimulated. In considering the effects of nicotine, it must be kept in mind that the drug may not only block transmission at the nerve endings of the ganglion and extrinsic nerves, but may act on the ganglion cell bodies themselves, causing a loss of their rhythmical activity.

In high concentrations of nicotine the effects vary, depending upon whether the heart gives a slow beat of normal form or whether it passes into a state of irregular intermittent tetani. In the former case, stimulation of the excitatory nerve may still be able to rouse the heart to more rapid beating, but in the latter case the excitatory nerve appears to be ineffective.

Frequency-intensity threshold curves for the excitatory nerves showed no significant changes after perfusion of hearts with nicotine 10^{-6} or 10^{-5}. Higher concentrations of nicotine could not satisfactorily be tested in this way, since the hearts generally failed during the lengthy process of determining the curve. Thus even though there is evidence that certain concentrations of nicotine enhance the response to excitatory nerve stimulation, it has not been possible to show any increased effectiveness of lower rates of excitatory nerve stimulation.

The augmentation of excitatory nerve effectiveness by low concentrations of nicotine may perhaps be comparable to the action of nicotine on vertebrate autonomic ganglia, where low concentrations of the drug augment the effect of preganglionic stimulation. In high concentrations, nicotine blocks the passage of impulses at vertebrate autonomic ganglia. In the crab heart nervous excitation has been blocked by nicotine in high concentrations, but only in cases where the heartbeat is of abnormal form. Is this failure of excitation due to a blocking of the excitatory nerve, or is it due to overstimulation of the heart ganglion? The abnormal form of the heartbeat suggests that the function of the heart ganglion has been impaired. If we accept this explanation, we cannot say whether or not transmission at the endings of the excitatory nerves is being blocked.

To sum up the results of this study of the effects of nicotine on the cardio-excitatory nerves, low concentrations of nicotine augment the action of these nerves, and high concentrations appear to block their effects. But, because of the likelihood that the heart ganglion is at least as sensitive to nicotine as are the excitatory nerves, it is not possible to say whether the block occurs at the endings of the excitatory nerves themselves or in the heart ganglion. The evidence so far obtained points to the presence of a "nicotine-like" cholinergic mechanism involved in nervous excitation of the crab heart, but it does not yet allow us to decide between the cardio-excitatory nerves and the intrinsic heart ganglion as possible sites of this mechanism. Perhaps both possibilities are true, since any effects of nicotine observed could be explained on such a basis. The methods employed in this work do not enable us to distinguish between nicotine-block of the cardiac ganglion alone and simultaneous nicotine-block of the excitatory nerve and the heart ganglion.

Effects of Eserine Upon Nervous Excitation and Inhibition

In the crabs used in the present work, eserine (physostigmine sulphate Merck) in concentrations of 10^{-6} or 10^{-5} was found to potentiate markedly the effects of low concentrations of acetylcholine. Hence such concentrations of eserine were considered sufficient to inactivate a significant amount of the cholinesterase in the heart when perfused for 15 minutes or more. Such low concentrations of the drug do not, by themselves, visibly affect the heartbeat.

The effects of eserine on nervous inhibition

Eserine in concentrations of 10^{-6} to 4×10^{-5} did not augment the action of the cardio-inhibitory nerves when tested by inductorium stimulation, thus agreeing with results previously obtained on *Panulirus* (Smith, 1940–41), nor could any effect of eserine upon frequency-intensity threshold curves be detected.

The effects of eserine on nervous excitation

Since the effects of nicotine on cardio-excitation have made it appear at least possible that the excitatory nerves are cholinergic, the action of eserine on the effectiveness of these nerves is of importance. However, no success was obtained in producing an augmentation of excitation by the application of eserine. Inductorium stimulation did not produce detectable results, while frequency-intensity threshold curves show little more. Eserine either has no effect, or it actually makes cardio-excitation more difficult to obtain. It should be recalled that the concentration of eserine used was sufficient to augment the action of acetylcholine on the crab heart, but that it did not by itself cause any stimulation of the heart. Under such conditions, Wiersma and Novitski (1942) have found eserine to augment the the effects of nervous excitation of the crayfish heart, as determined by a study of heart rate.

Effects of Acetylcholine Plus Eserine Upon Nervous Excitation and Inhibition

It has been found that a low concentration of acetylcholine chloride (10^{-7}) has a long-continued stimulatory action upon the heart of *Cancer* if it is potentiated with eserine 10^{-5}. Neither drug has any marked or persistent effect if perfused alone. Such a mixture of drugs has been used to determine the effects of acetylcholine upon the action of the regulatory nerves. But as in the case of erserine alone, "protected" acetylcholine in low, stimulatory concentrations has no consistent or significant effect either upon the inhibitory nerves or upon the excitatory nerves. Because higher concentrations of acetylcholine gave effects too strong to be maintained over the period necessary for the type of observations made in this study, they were not used.

Discussion

The lack of effect of eserine and acetylcholine upon the excitatory nerves would seem to speak against the possibility that these nerves are cholinergic. Yet there are indications that the failure to detect eserine potentiation of the effectiveness of these nerves is not conclusive evidence of their non-cholinergic nature. Welsh (1940–41) has reported that while eserine augments the effects of low concentra-

tions of acetylcholine on the isolated lobster heart, it fails to potentiate, or even lessens the effects of acetylcholine stronger than 10^{-5}. It is probable that cholinergic nerves release at their terminations acetylcholine in small amounts but in high, sharply localized concentrations; while cholinesterase has been shown to be greatly concentrated at the motor end plates in vertebrate skeletal muscle (Marnay and Nachmansohn, 1938; Nachmansohn, 1939). In the case of vertebrate autonomic ganglia, Brown and Feldberg (1936) have shown that eserine can potentiate the effect of preganglionic stimulation so effectively that the ganglion cells are soon paralyzed by the accumulation of acetylcholine unless submaximal preganglionic volleys and low frequencies of stimulation are employed. If such a situation exists in the crab, it is possible that eserine may allow the accumulation of mediator substance in depressant concentrations. In view of the evidence of Wiersma and Novitski (1942) that eserine augments nervous excitation of the crayfish heart, a restudy of this matter in the crab would be desirable, employing a technique by which heart rate, uncomplicated by amplitude changes, could be observed.

The inability of the excitatory nerves to tetanize the crab heart, although this state may be produced by direct electrical stimulation, acetylcholine, nicotine, etc., raises the question of whether or not the excitatory nerves act in the same way or at the same point as these other agents. Prosser (1943) has shown that the systole of the neurogenic heart of *Limulus* is initiated by synchronous activity in the cardiac ganglion, while tetanus results from a continued asynchronous discharge. During the course of the present work it has been observed that crab hearts depressed by nicotine or strychnine often exhibit a series of very irregular abnormal beats which, during recovery, regroup themselves to normal systoles, apparently by a return of the several cardiac ganglion cells to synchronous activity. The short tetani that may replace the normal systoles under the influence of nicotine or strychnine may, likewise, possibly be caused by asynchronous discharges of ganglionic cells. Direct electrical stimulation, acetylcholine, and other agents capable of tetanizing the neurogenic arthropod heart may do so by throwing the cardiac ganglion into a state of asynchrony, but it would seem that the excitatory nerves are capable of producing only more closely spaced bursts of synchronous ganglionic activity.

Since other evidence so far accumulated supports the view that the heart ganglion is cholinergic, and since the lack of effect of atropine upon normal heartbeat indicates that the ganglion does not exert a "muscarine-like" action on the heart, muscle, we may consider the possibility that the ganglion has a "nicotine-like" effect at the neuromuscular junction. It will be recalled that, in its action on different vertebrate tissues, acetylcholine has a variety of effects, which may be broadly classified into "muscarine-like" effects that can be blocked by atropine, and "nicotine-like" effects that can be blocked by high concentrations of nicotine. These classifications of acetylcholine action apply, strictly, to the reactions of vertebrate tissues, and it is possible that the reactions of invertebrate tissues do not fall into identical categories. Hence, the fact that the crustacean heart ganglion does not appear to exert a "muscarine-like" action upon muscle does not prove by elimination that its effects are fully comparable to the "nicotine-like" action of certain cholinergic nerves in vertebrates. For the present, however, we may consider that the cardiac ganglion transmits impulses to the heart muscle by a cholinergic mechanism having a somewhat "nicotine-like" effect.

If it is true that both the ganglion and the excitatory nerves are of the same pharmacological nature, this fact renders useless our attempts to demonstrate a nicotine block of the excitatory nerves. As pointed out above, the ganglion seems more sensitive to nicotine than are the excitatory nerves. Paralysis of the ganglion removes an essential link in the chain of impulses which pass from regulatory nerve to heart muscle in nervous excitation. With the ganglion out of action, the present methods do not reveal whether or not the excitatory nerves are blocked by high concentrations of nicotine, but the positive evidence so far obtained points to a cholinergic "nicotine-like" action of the cardio-excitatory nerves upon the heart ganglion.

Summary

1. The heart of *Cancer irroratus* has been perfused *in situ*, the beat recorded mechanically, and the inhibitory and excitatory nerves stimulated while the heart was perfused with various drugs.

2. Nervous inhibition of the heart induced by stimulation of the inhibitory nerves is usually total with no inhibitory after-effect.

3. Nervous excitation of the heart induced by stimulation of the excitatory nerves frequently shows a stimulatory after-effect.

4. The cardio-inhibitory nerves appear to be more effective than the excitatory nerves, but each type of nerve can modify to some extent the effectiveness of the other.

5. The excitatory nerves are effective when stimulated at frequencies ranging from 200–300 per second down to 10 per second or less.

6. The inhibitory nerves produce total inhibition when stimulated from 15–20 to 100–200 times per second. At frequencies extending above and below this range, partial inhibition may be obtained.

7. Strychnine, in concentrations of 1 : 2,500, blocks reversibly the action of both excitatory and inhibitory nerves.

8. Atropine is without effect upon excitatory and inhibitory nerves, as well as upon the heart ganglion in concentrations sufficient to abolish the action of applied acetylcholine.

9. Nicotine, in low concentrations, augments the effectiveness of the excitatory nerves as well as of the heart ganglion.

10. Nicotine in high concentrations may block the passage of impulses from cardiac ganglion to muscle; hence it could not be determined with certainty if nicotine blocks the excitatory nerves.

11. Neither eserine alone, nor with acetylcholine in stimulatory concentrations, augmented cardio-inhibition or excitation. It is suggested that the lack of effect of eserine upon the excitatory nerves deserves restudy.

12. It is probable that the excitatory nerves are cholinergic, and exert a somewhat "nicotine-like" effect upon the heart ganglion, which in turn exerts a "nicotine-like" action at the neuromuscular junction.

13. No light has been thrown on the pharmacological nature of the cardio-inhibitory nerves.

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